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# PROCEEDINGS

# TECHNOLOGY TRANSFER CONFERENCE

December 8 & 9, 1986

THE SHERATON CENTRE
TORONTO, ONTARIO,
CANADA

## Part A
## Air Quality Research

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# PROCEEDINGS

## TECHNOLOGY TRANSFER CONFERENCE

December 8 & 9, 1986

The Sheraton Centre

External Research Projects

## PART A
## AIR QUALITY RESEARCH

Organized by
Research Advisory Committee

Sponsored by

Corporate Resources Division
Ministry of the Environment
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CANADA

HAZARDOUS CONTAMINANTS
COORDINATION BRANCH
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## Goal Statement

"To achieve and maintain a quality of the environment - including air, water and land - that will protect human health and the ecosystem and will contribute to the well-being of the people of Ontario."

## ACKNOWLEDGEMENTS

The Conference Committee would like to thank the speakers and organizers for their valuable contributions.

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## INTRODUCTION

The Ontario Ministry of the Environment holds its annual Technology Transfer Conference to report and publicize the progress made on Ministry-funded projects. These studies are carried out in Ontario universities and by private research organizations.

The papers presented at the Technology Transfer Conference held in December 1986 are presented in Conference Proceedings. These Proceedings are divided into five parts corresponding to the conference sessions dealing with:
Part A: Air Quality Research ;
Part B: Water Quality Research;
Part C: Liquid & Solid Waste;
Part D: Analytical Methods; and
Part E: Environmental Economics.

This part is a collation of those papers presented at Session A of the Conference.

For further information on any of the projects, the reader is kindly referred to the Principal Investigators or to the Research Management Office at (416) 965-5788.

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# AN OVERVIEW OF ALBERTA ENVIRONMENT'S ATMOSPHERIC DEPOSITION PROGRAM

H.P. Sims, G.A. Singleton, H.S. Sandhu, and B.L. Magill

## 1. INTRODUCTION

Atmospheric emissions of pollutants, their deposition, and potential long-term detrimental effects on the environment have not only become a serious scientific issue, but a focus of significant public concern in Alberta. Nevertheless, industrial development is dictated by economic opportunity and cannot wait for long-term research projects to provide all of the answers to possible problems. In many cases, we must rely on judgement and external experiences to allow promotion of development goals without sacrificing critical components of our environment. We are aware that our perspectives of the relationship between environmental quality and industrial development are diverse and vary from region to region. However, the concern for environmental quality has never been higher in our history than at present, and determined efforts by the public to have an influential role in the decisions that affect the fate of our environment are rightfully increasing.

With the use of emission abatement strategies, local ground level concentrations have decreased in the vicinity of new stationary sources. However, the potential for long-term, progressive, subtle impact on the environment remains. A number of issues and concerns have been identified in documents published by Alberta Environment (Appendix I). Relative to Alberta, our knowledge regarding medium and long-range transport of air pollutants/acid rain and the environmental and human health effects of long-term, low-level emissions is presently limited. Research and monitoring/surveillance programs need to be expanded and implemented to overcome our knowledge deficiencies. To facilitate this, Alberta Environment established three research programs (Departmental Acid Deposition; Air Quality; and Environmental Contaminants).

## 2. DEPARTMENTAL ACID DEPOSITION PROGRAM

Acid-forming emissions, such as oxides of sulphur and nitrogen, can be transported from a few to thousands of kilometers. Because of the dramatic deleterious environmental effects observed at Sudbury, Ontario; Trail, British Columbia; and in the vicinity of many point sources in the United States, it was generally believed that emissions resulted mainly in local environmental impacts. However, scientific evidence has been obtained to show that transport of these emissions occurs over long distances, such as from the Ohio River Valley through the northeastern United States into eastern Canada, from the United Kingdom into Norway, and from the Ruhr Valley in West Germany into Sweden. Similarly, emissions originating in Alberta are transported to areas downwind of major sources, where ecosystems may be sensitive to acid deposition (Sulphur Gas Emissions in the Boreal Forest: The West Whitecourt Case Study, 1978; Environmental Sulphur Research in Alberta: A Review, 1980). The present and potential impacts of these emissions are not well understood. Some of the concerns are: (a) acidification of surface waters and the potential consequences for aquatic biota, including fish; (b) acidification of soils and their influence on the total watershed, vegetation and aquatic systems; (c) direct effects on agricultural crops and forest tree species; and (d) direct and indirect effects on human and animal health.

### 2.1 OBJECTIVES OF THE PROGRAM

The overall objective of the acid deposition program is to design, co-ordinate, and implement a research and monitoring program concerning acid-forming emissions and their ecological effects in Alberta. The program will provide scientific information for evaluating and reviewing Alberta's current air quality standards, emission guidelines, and regulatory controls with respect to socio-economic and environmental consequences of atmospheric pollutants to satisfy the concerns of Albertans.

Specific objectives of the Departmental program are to:

1. Undertake routine monitoring of air, water, soils, and vegetation to determine short- and long-term trends in environmental quality;

2. Characterize sensitive environmental components and develop methodologies to (a) identify and/or predict environmental deterioration caused by acid-forming substances in Alberta; and (b) determine when environmental deterioration begins and whether or not the effects are reversible;
3. Characterize, separate, and quantify the contributions of emissions, from local sources and from the medium- and long-range pollutant transport, to environmental effects of acidification;
4. Review, co-ordinate and implement a research program concerning acid-forming emissions and their short- and long-term environmental and health effects;
5. Determine the socio-economic impacts of acidification and develop a scientific basis and approach for preventive and/or mitigative strategies and environmental protection taking into account the socio-economic factors;
6. Provide support to and integration of the research, monitoring, and other activities of the Departmental Committee on Acid Deposition, Alberta Government/Industry Acid Deposition Research Program (ADRP), the Western and Northern Canada LRTAP Technical Committee (W&NC-LRTAP), and other agencies.

## 2.2 PROGRAM COMPONENTS

The Acid Deposition Research Program is comprised of five distinct but integrated components:

1. Alberta Environment's Acid Deposition Effects Research Program;
2. The Alberta Government/Industry Acid Deposition Research Program;
3. Western and Northern Canada LRTAP;
4. Departmental Monitoring Program; and
5. Inhalation Toxicology and Animal Health Program.

Each of these components is discussed in the ensuing sections.

## 2.3 ALBERTA ENVIRONMENT'S ACID DEPOSITION EFFECTS RESEARCH PROGRAM

Forest decline evident in Europe and in eastern North America has been attributed to acid deposition, although scientists still debate which of several mechanisms may be responsible for the decline. In Alberta, government and the public are concerned whether or not a similar decline will occur in Alberta's forests as a result of acid deposition. Alberta Environment's acid deposition effects research program was developed to assess long-term environmental impacts of industrial developments in the oil sands region of northeastern Alberta on forest and aquatic ecosystems. Research has been co-ordinated under the auspices of the Alberta Oil Sands Environmental Research Program (AOSERP).

### 2.3.1 Program Development 1975 to 1985

The initial phase of the program (1975 to 1980) accumulated baseline information required to quantify and characterize existing environmental conditions, primarily dominant soil and vegetation types. This baseline information was to be used to identify ecosystems most likely to be impacted by acid deposition and to assess the impact of industrial development on these ecosystems.

Air quality and meteorological networks were established to collect data on local air mass trajectories and climate. These data were used in the development and verification of an air quality model capable of predicting the ground level concentrations of pollutants based on varying meteorological conditions and emission levels. The model was used to delineate areas of maximum $SO_2$ loading, and therefore subject to potential acidification and long-term environmental damage.

To further address the environmental impact of industrial development, precipitation chemistry monitoring networks were established. The accumulated snowpack and event rainfall were extensively sampled and analyzed to determine: (1) trends in the ionic and particulate concentration of precipitation resulting from industrial emissions, and (2) deposition patterns and spatial variability of wet deposition pollutant loadings.

Although baseline information was collected on the sensitivity of terrestrial and aquatic ecosystems to pollutant deposition, strategies had not been implemented to ensure the interdisciplinary, integrated approach needed to understand the complex interrelationships between atmospheric and biological processes. In a review of the research and monitoring activities undertaken during 1975 to 1980, research deficiencies were identified and future research recommended. Specific recommendations were:

1. To characterize and quantify regional and local source emissions to ascertain their relative contribution to environmental acidification;
2. To integrate episodic air quality data with receptor response in order to (a) characterize sensitive environmental ecosystems and (b) develop methodologies to identify and/or predict measurable short- and long-term effects of acidic or acidifying substances on terrestrial and aquatic ecosystems;
3. To develop a scientific basis and approach for preventive strategies and long-term environmental protection/management; and
4. To co-ordinate departmental research on acid deposition with other private and public agencies in Alberta and the rest of western Canada.

From 1980 to 1985, the program concentrated on addressing these research deficiencies, especially characterizing and quantifying the ecological effects of acidic and acidifying substances. Considerable effort and resources were expended to develop a more thorough understanding of the atmosphere-biosphere interface. Research consisted of field and laboratory investigations of plant responses to emitted pollutants. Specific projects were geared to the accurate and frequent measurements of pollutant concentration occurrences and their interface with physiological, phenological and productivity responses in vegetation species. A second major interface, between soil nutrients, microbiological processes and vegetation was also being studied.

With respect to atmospheric research, regional surveys to sample and chemically analyze wet deposition were continued. Wet deposition, however, is only one of two major mechanisms in the transfer of pollutants from source to the ecosystem receptor. Air pollutants released into the atmosphere are deposited continuously and cumulatively through both dry and wetfall processes. Methodologies and instrumentation technology for measuring dry deposition to a forest canopy were developed and implemented on an experimental basis. However, more evaluation and research are required before dry deposition monitoring techniques can be employed on a routine operational basis. In conjunction with these studies, research to determine the transport, dispersion, and transformation rates of sulphur and nitrogen oxides emissions were undertaken to facilitate the investigation of the medium- to long-range transport of acid-forming emissions from a major emission source in the oil sands area.

A summary report for research and monitoring work carried out in the oil sands area between 1975 to 1985 is in preparation.

In the 10-year period 1975 to 1985, research demonstrated clearly the high degree of natural variability in ecosystem parameters, especially in forest ecosystems, which would make it difficult to detect subtle changes in the ecosystem occurring as a result of low acid-forming emission levels. Therefore, it became clear that a new strategy was required for evaluation of acid deposition effects in the oil sands area.

### 2.3.2 The New Five-Year Research Plan

Effects research can only be done meaningfully in the oil sands area using long-term monitoring techniques in which biological variability is statistically accounted for. Before embarking on any additional new research efforts in the northern forested areas, Alberta Environment felt it was necessary to undertake a detailed review of the existing research studies and make use of the world literature reviews conducted in the Government/Industry Acid Deposition Research Program. The Department has completed the review and produced a new strategy to develop biomonitoring indices which will be used to monitor effects of low levels of acid-forming emissions over the medium- and long-term.

A new five-year plan for research in the oil sands area was initiated in 1986/87. The five-year research needs of this program are summarized in **Table 1**. This new program, which is described in the following sections, includes the following activities and will guide acid deposition research in northern forested areas of Alberta until at least 1991:

1. Evaluation of biomonitoring indices for terrestrial and aquatic systems;
2. Selection of biomonitoring methods and compilation of a data base over a deposition gradient of acid-forming emission; and
3. Investigation of data to develop empirical relationships between emissions and biomonitor response.

The effects research program is designed to address the question; is there measurable environmental damage due to acidification which can be monitored over the medium and long-term? If ecosystem damage is occurring, the hypothesis is that the pollutant loading and some corresponding quantitative measurement of damage will vary significantly along the deposition gradient. In order to identify an appropriate pollution gradient and determine deposition inputs for effects research, atmospheric research will determine sources, distribution, and deposition characteristics of the existing acid-forming emissions in the oil sands area. A monitoring program will be designed to determine the (1) horizontal and vertical distribution of atmospheric gaseous and particulate matter within and above the forest canopy, and to the soil and aquatic ecosystems; (2) wetfall deposition at canopy height and ground level to provide information on throughfall and available input to the soil and groundwater; and (3) microclimate of the receptor area.

Source apportionment studies and projects to upgrade emission inventories by collecting information on fugitive and low-level emissions are proposed in order to quantify and characterize the total emission burden resulting from Syncrude, Suncor, and other anthropogenic sources in the oil sands area. This will enable Alberta Environment to assess the environmental impacts of present and future oil sands development.

Table 1. Five-year research needs for Alberta Environment's Acid Deposition Research Program.

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1. Organized evaluation and synthesis of existing information on atmospheric processes, deposition and receptor responses, as they relate to Alberta.

2. Description of regional (provincial) scale meteorology and atmospheric processes.

3. Characterization of the physics and chemistry of selected Alberta point source plumes, with simultaneous ground level measurements.

4. Characterization and quantification of atmospheric deposition of beneficial and deleterious chemical constituents into Alberta ecosystems.

5. Identification and qualitative and quantitative characterization of sensitive receptors and processes related to acid deposition (laboratory and field).

6. Development of numerical methodologies to correlate atmospheric processes and pollutant deposition to effects on total ecosystems (i.e., receptor response), using an integrated approach. This should evolve into predictive capabilities to forecast air pollutant-induced ecosystem impacts.

7. Characterization and quantification of the effects of atmospheric pollutants on human/animal health.

8. Interpretation of program results to determine socio-economic and environmental consequences and mitigation strategies which will assist decision makers in the development of public policy and regulatory guidelines and controls to ensure and maintain environmental quality and human/animal health.

Biomonitoring indices selected for impact assessment research will have to be evaluated first for their spatial and temporal variability. Vegetation research will involve testing the variability of biological indicators of acidification, such as needle length, needle retention, and seed viability. Provided that real impact-associated differences can be discriminated, the appropriate biomonitoring indices will be incorporated into a monitoring program and tested on a regional basis. A further restraint is that the monitoring indices must be feasible for use in medium-term (up to 25 years) programs.

Although some routine monitoring of soil parameters may be done for site characterization purposes, detailed monitoring of a wide range of soil parameters for detection of impacts in the medium-term is not considered advisable. Rather, soil effects research will concentrate on the identification of those soil components that buffer against long-term changes resulting from acid-forming emissions. A model would be developed to determine the buffering capacity of sensitive forest soils, and what effects current and potential long-term loadings of acid-forming substances have or could have on soil chemistry. Predictions would be made as to how long, at assumed loading levels, it would take to cause major changes in soil chemistry and especially nutrient cycling capabilities.

An investigation will be made of the modelling techniques available for translating pollutant loading measurements or predictions to changes in water chemistry. Modelling techniques will be adopted to provide the following information:

1. Area loading (atmospheric) translated into predictable changes in water chemistry;
2. Time frame for observable changes in water chemistry; and
3. Rate at which acidification and changes in water chemistry would proceed.

## 2.4 GOVERNMENT/INDUSTRY ACID DEPOSITION RESEARCH PROGRAM

The formation of the Government/Industry Acid Deposition Research Program (ADRP) in 1985 is the result of a 10-year evolution to study and address the environmental consequences of acid-forming emissions on our environment. Public concern regarding effects of emissions from sour gas wells in southwestern Alberta on agricultural productivity and human health has been an integral part of this evolution. ADRP is an $11 million undertaking by the Province, industry (Canadian Petroleum Association, Alberta Power, Edmonton Power, Transalta Utilities) and the Energy Resources Conservation Board. Research is focussed on the agricultural effects of two principal gas groups, oxides of sulphur and nitrogen, and on human health effects. The research on agricultural effects is jointly funded by Alberta Environment, industry (Canadian Petroleum Association, Alberta Power, Edmonton Power, Transalta Utilities), and the Energy Resources Conservation Board. The human health research was completely funded by Alberta Social Services and Community Health.

The first critical phase of the agricultural research component, which spans a period of 32 months, will involve:

1. An initial assessment of the potential impact of emissions on Alberta's environment including local and regional air quality assessments. Three sites have been selected to monitor air quality parameters; a background site (Fortress Mountain, 100 km west of Calgary) to monitor air quality entering the province, and two sites in the study area (Crossfield, 30 km north and northeast of Calgary) representative of downwind and regional air quality;
2. An inventory of sulphur oxide and nitrogen oxide emissions in Alberta; and
3. A world literature review in the biological sciences on the effects of acid deposition on vegetation, soils, and surface and groundwaters. This will provide a comparative basis for a 'first order' assessment of the current status and future potential for acidification in Alberta.

The last two tasks are nearing completion and reports should be available in 1987.

The human health component of the program was designed to address the specific and long-standing health concerns of residents in the southwestern towns of Twin Butte, Hill Spring, Mountain View, and Glenwood (Index Area) to determine if residents of the Index Area are healthier or less healthy than other Albertans. Some residents have had long-standing health complaints which they attribute to sour gas plant operations. Two other communities served as controls - one exposed to "pollution" (Didsbury, Carstairs, Crossfield, Irricana) and one not exposed (Stirling, Raymond). A comprehensive health assessment was undertaken using questionnaires and physical examinations. Over 3600 residents were medically examined in the summer of 1985 by the team of doctors headed by Dr. W.O. Spitzer of McGill University. While the study did not deal with cause/effect relationships, it established that the health of residents in the Index Area was not significantly different from other Alberta communities. The human health study, the Medical Diagnostic Review, was completed in June 1986 and the final report is currently being reviewed.

In addition to the Medical Diagnostic Review, an occupational epidemiology feasibility study, which will focus on the occupational work force of the sweet, sour, and thermal gas industry, will attempt to determine whether acute or chronic exposure to sour gas or its by products has significantly affected the health of the workers. To ensure the scientific integrity of the program, scientific advisory boards comprised of international environmental and medical scientists provide advice on and peer review of the program. In recognition of the value of public views of the program, a public advisory board has been formed to facilitate involvement from and communication with the general public and environmental interest groups.

## 2.5 WESTERN AND NORTHERN CANADA LRTAP PROGRAM

The departmental program provides the Alberta focus for the Western and Northern Canada Long Range Transport of Air Pollutants (W&NC-LRTAP) federal/interprovincial program which is investigating the medium- and long-range transport of air pollutants and their possible environmental effects.

The national and provincial governments of Canada have a tradition of joint action on environmental issues, resulting in cost-effective research and monitoring programs and a uniform approach to problems regardless of provincial boundaries. The acid rain program in western and northern Canada is a good example.

British Columbia, Alberta, Saskatchewan, Manitoba, Northwest Territories, and Canada first held joint consultations on the acid rain issue in 1980. By 1982, they, as a consequence of their commitment to undertake research and monitoring programs to protect sensitive ecosystems from potential acidification, signed an agreement for a three-year research program covering the period 1982 to 1985. The program's objectives included identifying current conditions in western Canada and providing information necessary to develop appropriate management strategies. The six program priorities and the major accomplishments attained during this period are delineated in the ensuing points:

1. Maps of aquatic sensitivity
    a. methodologies/criteria for determining sensitivity were developed; and
    b. preliminary maps completed.
2. Maps of soil and geology sensitivity
    a. methodologies/criteria for determining sensitivity were developed; and
    b. preliminary maps completed.
3. Monitoring of background precipitation
    a. standard protocols developed; and
    b. monitoring network expanded to 62 stations.
4. Modelling of atmospheric deposition patterns
    a. long-range models applied to western Canada and deposition maps produced; and
    b. mesoscale modelling workshop held to review and recommend mesoscale models for application in western Canada.
5. Emission inventories of sulphur dioxide and nitrogen oxides
    a. inventories regularly updated; and
    b. standardization of inventory data base pursued.

6. Monitoring of surface waters and soils in data sparse areas
    a. data base expanded; and
    b. standard water sampling methodologies pursued.

In 1984, W&NC-LRTAP agreed to continue co-operative action on a strategy for acid deposition to protect sensitive ecosystems from potential acidification. The identified research priorities for the subsequent three-year program 1985 to 1988 provide for: (1) the continuation and tailoring of the monitoring activities for parameters considered to be of special significance in western Canada, (2) the establishment of deposition standards suitable for the sensitivity of our environment, and (3) the investigation of deposition models and control models which will allow evaluation of the impact of individual sources, and the most cost-effective manner of implementing a control program. These priorities are:

1. Monitoring of air, rain, and snow;
2. Emission inventory update;
3. Deposition criteria/target loading for western Canada;
4. Medium-range transport/deposition models;
5. Background ion concentrations and the influence of dust on precipitation chemistry and total deposition loadings;
6. Dry deposition measurements;
7. Monitoring of sensitive surface waters and terrestrial environments; and
8. Evaluation of impact and assessment of control technology.

Projects undertaken by the Department to address the high priority needs identified by W&NC-LRTAP are highlighted in the Western and Northern Canada LRTAP annual reports.

## 2.6 DEPARTMENTAL MONITORING PROGRAM

The Department is involved in an extensive monitoring program of soil, water, air, and precipitation quality as part of its mandate to protect the environment. Monitoring of pollutants is conducted by line divisions within Alberta Environment to assist the Department in setting emission standards and determining compliance with existing regulations.

### 2.6.1 Precipitation Quality

The precipitation quality monitoring program was initiated in 1978 by the Air Quality Control Branch, Pollution Control Division, Alberta Environment. The objective of the program is to monitor the chemical characteristics of rain and snowfall which are representative of different regions in Alberta. The parameters of particular importance are the acidity or pH of precipitation and the sulphate and nitrate deposition rates which are directly related to the acid rain issue.

From 1978 to 1984, there were six provincial precipitation stations in the network. They were located at Beaverlodge, Calgary, Edmonton, Red Deer, Suffield, and Whitecourt. During 1985, three additional stations at High Level, Cold Lake, and Kananaskis were put into operation. It is intended that these three stations will provide additional information about the incoming background chemical contributions to precipitation events.

Besides the nine-station provincial network, the federal government operates five precipitation quality monitoring stations in Alberta under the CANSAP program (Canada Network for Sampling Precipitation). Their stations are located at Coronation, Edson, Fort McMurray, Lethbridge, and Rocky Mountain House. All stations in both networks collect monthly samples using automatic rain and snow collectors. Their data are directly comparable. Measurements of pH and major ionic concentrations are made on each accumulated monthly sample. The quality of the precipitation is assessed based on the different parameters: acidity from pH, acid-forming emission contributions from sulphate and nitrate concentrations, dust-related contributions from calcium and magnesium concentrations, and salt contributions from sodium concentrations.

In 1986, the CANSAP Program was replaced by the CAPMON Program which collects precipitation on a daily event basis rather than monthly. The only CAPMON station in Alberta, at this time, is located at Esther.

The monitored data from the federal network and the provincial network supplement each other to increase the coverage in Alberta. The locations of all stations in Alberta are illustrated in **Figure 1**. The accumulated data base allows the detection of any significant changes in

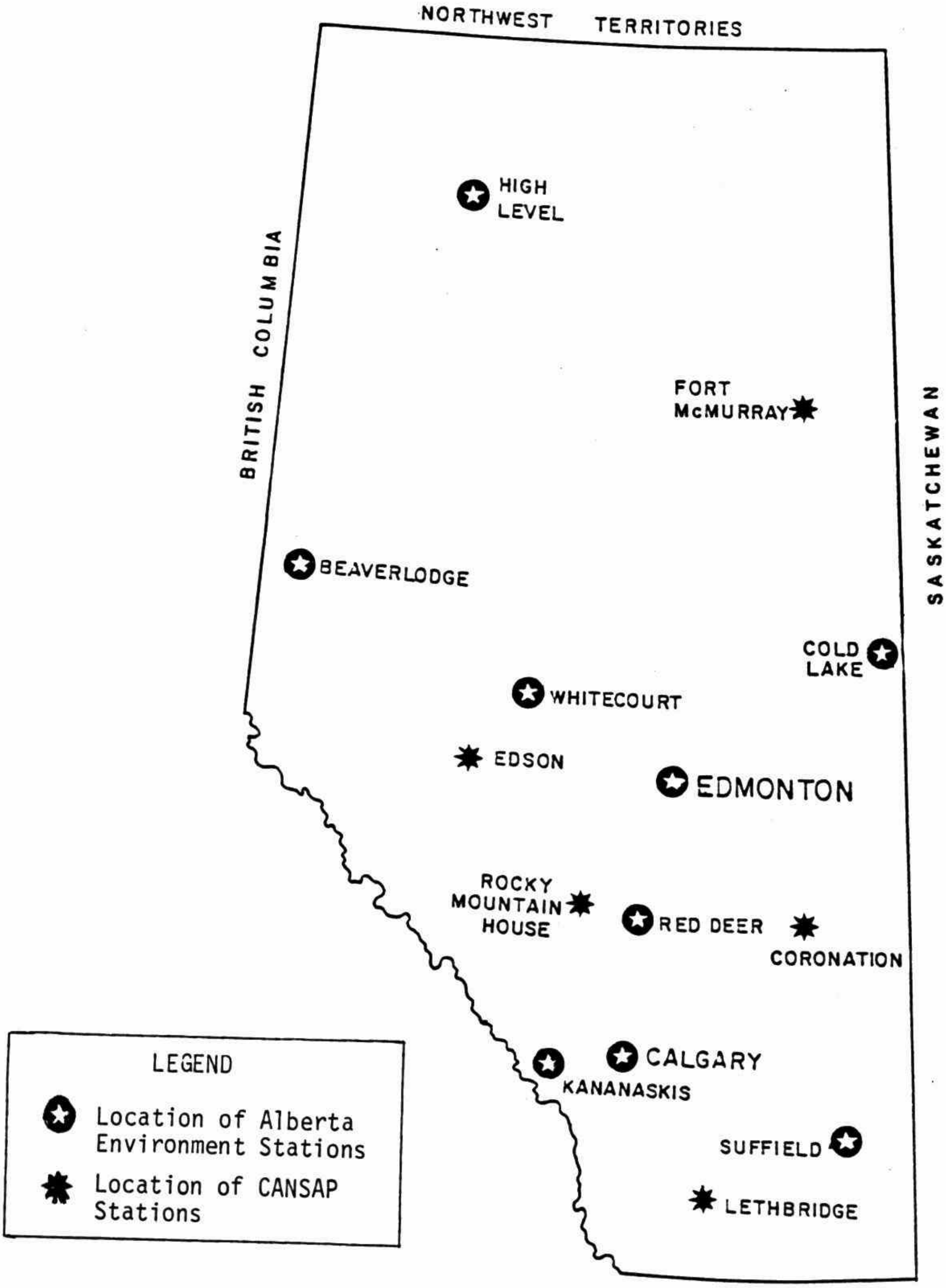

Figure 1. Location of precipitation quality monitoring stations in Alberta.

precipitation quality that may occur as a result of industrialization or long-range transport of the acid-forming pollutants.

Annual reports summarizing the data from the federal and provincial stations are published by the Air Quality Control Branch, Pollution Control Division, Alberta Environment.

### 2.6.2 Exposure Stations

The Exposure Station Program consists of 2112 total sulphation and 1276 hydrogen sulphide static exposure stations. These monitors are primarily located in and around urban centres and industrial areas of the province and are used to identify trends. Chemical analysis is done by the Alberta Environmental Centre (Vegreville), and the results are expressed as $SO_3$ equiv. mg/day/100 $cm^2$.

### 2.6.3 Soil Monitoring

Long-term soil acidification monitoring in Alberta carried out by Earth Sciences Division, Soil Protection Branch, began in September 1981. Monitoring plots have been established at eight different areas of the province (Rocky Mountain House, Fort McMurray, Twin Butte, Cold Lake, Esther, High Prairie, Devon, and Bruderheim) between 1981 and 1984 (Figure 2). The reason for establishing this program was to provide an early warning of the potentially adverse effects of atmospheric acid deposition on Alberta's soil resource. The objective of this program is to detect subtle changes in soil properties indicative of potentially significant long-term changes in the ability of soil to sustain plant growth. All sites will be resampled once every four years. The two principal components of the monitoring program are the field sampling and the laboratory analysis.

Soils are the only components routinely monitored at these sites. It is planned to integrate total monitoring efforts (i.e., vegetation, air quality, water quality, and soil) at selected sites in the near future. A concerted effort will be made to combine activities of the relevant provinces and federal agencies.

An initial assessment of the sensitivitiy of Alberta soils to acid deposition has been completed. Maps depicting (1) potential of soils and

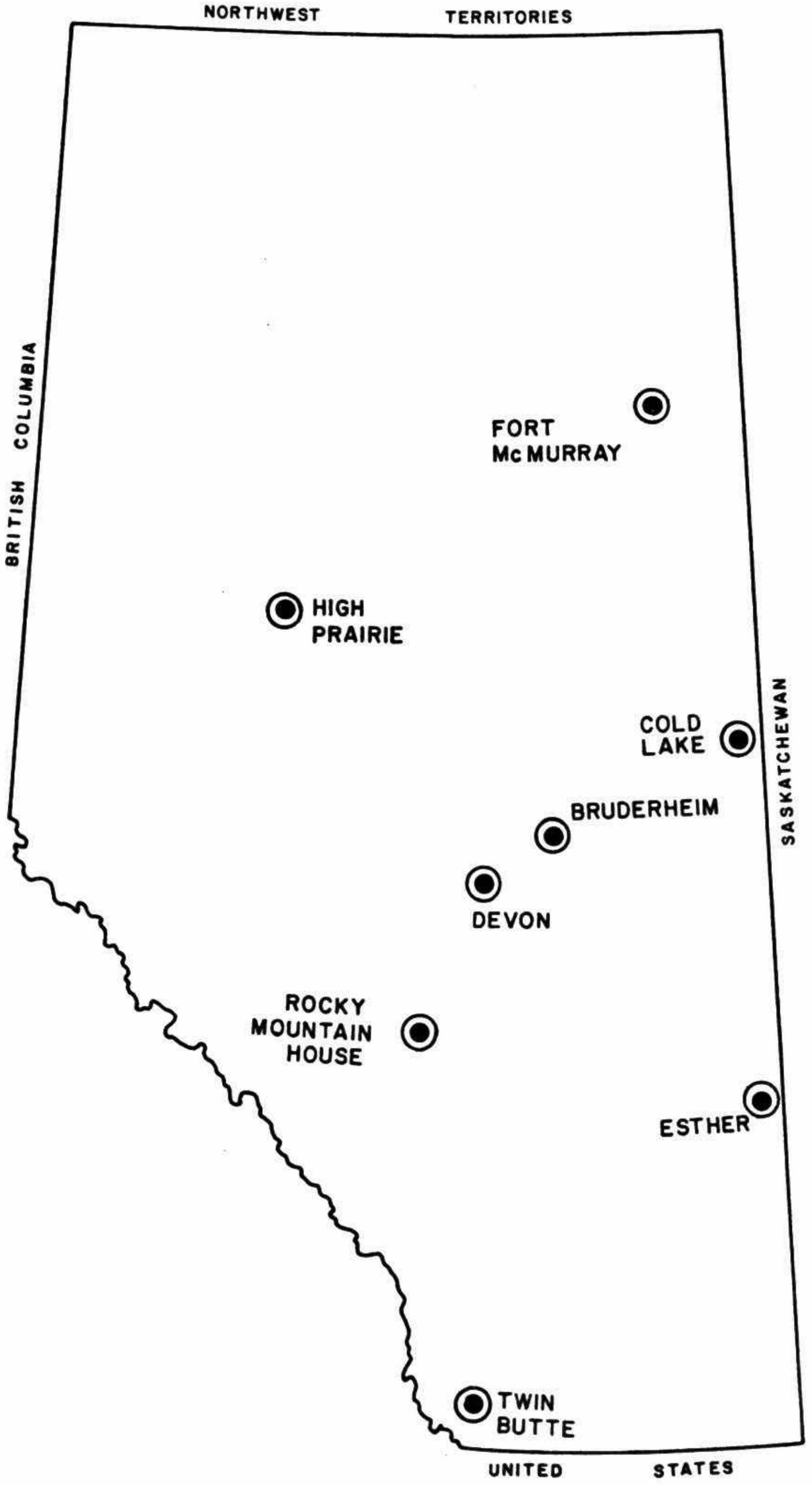

Figure 2. Long-term soil acidification monitoring sites.

geology to reduce acidity of incoming acid deposition, and (2) sensitivity of soils to acidic inputs have been prepared.

2.6.3.1 Field sampling. Five characteristics were considered important to the selection of monitoring sites:

1. General areas selected for sampling were those identified as areas receiving maximum amounts of acid deposition. These were areas downwind from major sour gas plants, oil sands plants, coal-fired generating plants and heavy oil extraction plants. Care was taken, however, to locate in areas beyond which the localized effects of elemental sulphur dusting could have been an additional source of acid deposition.
2. The most sensitive soils were sampled in each area.
3. Plot areas were located on sites that were as homogeneous as possible with regards to soil characteristics.
4. Undisturbed native soils were selected. Six of the areas are forested and two are on native grassland. Cultivated areas were excluded because of the potential for soil acidification from farm management practices (e.g., fertilizers).
5. Each sampling site was secured, along with a buffer area, by registration with the Land Titles Office. As a precautionary measure, paired plots were established at each site.

The first two characteristics were those considered essential factors for an early warning system. The next two characteristics are important to the detection of subtle changes. The final characteristic is essential to allow for long-term monitoring.

2.6.3.2 Laboratory analysis. Due to the many different effects of soil acidification, the laboratory analysis program is designed to measure changes in the status of a broad variety of soil chemical properties. Elements chosen for measurement include hydrogen, calcium, magnesium, potassium, sodium, aluminum, iron, manganese, sulphur, nitrogen, carbon, and phosphorus. Soil testing procedures were selected on the basis of being widely accepted and appropriate for measuring the properties of acidic soils. Particular care has also been taken to reduce the introduction of variability due to the laboratory analysis program.

### 2.6.4 Water Quality Monitoring

The Water Quality Control Branch of the Pollution Control Division maintains a fixed, long-term monitoring network on 11 river locations and four central Alberta lakes (Ethel, Baptiste, Nakamun, and Wabamun). All sites are sampled monthly, and analyzed for a wide range of chemical constituents and indicators of acidity.

An initial province-wide assessment of the sensitivity of Alberta lakes to acid deposition has been completed.

This analysis has identified a number of areas in the northern upland regions, the Canadian Shield area, and the Rocky Mountain National Parks which are potentially sensitive. Accordingly, our long-term monitoring network will be expanded to incorporate representative lakes in areas which may be subject to increased atmospheric loading of acid-forming substances. The expansion will occur in 1986 or 1987, subject to budgetary conditions.

## 3. ANIMAL HEALTH

The Alberta Environmental Centre is undertaking a study on the health effects of sulphur gases. The objective is to determine the effects of toxic or potentially toxic gases, vapours, particulates, and aerosols on animals and, by extrapolation, on humans. Current studies include the characterization of a 69 L inhalation exposure chamber, commissioning of a new 359 $m^2$ inhalation toxicology containment facility, studies on the acute toxicity of hydrogen sulphide, in vitro studies and techniques for evaluation of adverse effects of pneumotoxicants, and implementation of an inhalation exposure system using 2 $m^3$ exposure chambers, and field investigations of possible effects on livestock.

## 4. DEPARTMENTAL AIR QUALITY AND ENVIRONMENTAL CONTAMINANTS PROGRAM

The major emphasis of Alberta Environment's deposition research program has focussed primarily on the issue of 'acid rain'. However, the effect of other toxic pollutants/contaminants from natural and anthropogenic sources on health and the environment are of major concern provincially, nationally, and internationally. With our increasing population base and economic development, the increase in pollutant emissions and their secondary or tertiary reaction products are important contributors to the degradation of air quality. Further, today with nearly 50 000 chemicals utilized in Canada, it is impossible to escape exposure at some level to chemicals in the work place, at home, in the air, and in food products. Environmental contamination has been increasing steadily within this century. The primary concern is that although toxic pollutants and contaminants may be emitted and dispersed at low concentrations, they may persist and accumulate in the environment and bioaccumulate in the food chain. These contaminants impact the environment through industrial effluents/air emissions, spills, spray programs, and natural releases. Because of their potential impacts, Alberta Environment has developed research programs to address issues related to air quality and environmental contaminants.

### 4.1 AIR QUALITY

In recent public surveys in Alberta, air quality has been defined as a leading environmental concern; the issue ranked fourth in Alberta in 1981, and first in 1982 and 1983. The assessment and management of ambient air quality requires research and monitoring to understand the quantitative relationships among background air quality, emissions, ambient air quality, atmospheric transport/dispersion/diffusion, chemical transformation, deposition, and probable effects.

Specific objectives of the air quality program are:

1. Monitoring
    - a. To characterize and quantify atmospheric pollutants from various sources;

   - b. To quantify the contributions of local and regional sources to air quality degradation; and
   - c. To determine short and long term trends in air quality.
2. Effects
   - a. To develop methodologies to identify and predict environmental damage and health effects caused by atmospheric pollutants; and
   - b. To determine the socio-economic impacts.
3. Management Strategies
   - a. To determine what are the best possible management strategies and regulatory controls required to prevent or mitigate degradation of air quality.

Research in three high priority areas has been proposed.

### 4.1.1 Flaring

Historically, flares have been used in the oil, natural gas, refining, and petrochemical industries to dispose of gases in a safe manner. However, the efficiency of flaring in the disposal of reduced and oxidized sulphur compounds and other toxic compounds has been a concern for several years. Odour, smoke, air quality, and health problems in the vicinity of these industrial activities have been attributed to flaring operations. The real or perceived risks associated with these activities has given rise to environmental and health concerns which must be adequately addressed.

In 1986, a Government/Industry Consultative Committee on Flaring was established by Alberta Environment. Based on a review of relevant studies pertaining to flaring, the committee felt that further investigation was necessary to confirm and define more clearly the environmental and health impacts associated with flaring.

The first initiative identified by the committee was a two part study to document and compare the design and operating practices of flaring operations in Alberta against the technology available through suppliers and designers. These studies are being funded by the federal and provincial governments.

### 4.1.2 Fugitive and Uncontrolled Emissions

An area of growing concern is the environmental and health effects of fugitive and uncontrolled emissions. In most industrial operations, only the main source of pollutants are routinely monitored. However, all facets of heavy oil recovery and production operations are potential sources of atmospheric emissions which may adversely affect ambient air quality and the environment. These emissions have not been characterized or quantified. Although these emissions may be emitted and dispersed at low concentrations, they may persist and accumulate in the environment and food chain. Also nitrogen oxide and hydrocarbon emissions are precursors to photochemical oxidant pollution which can damage forest and agricultural ecosystems. A more quantitative assessment is needed if a thorough investigation and understanding of the effects is to be obtained. Information from the proposed research will be used to ascertain the need to and the appropriate way to control emissions to avoid undesirable effects.

### 4.1.3 Urban Air Quality

Urban air quality has been identified as a leading environmental concern. Studies have indicated that the potential for serious pollution problems exists in both Calgary and Edmonton, major urban centres in Alberta. The efficient management of urban air resources and the planning and design of environmental legislation, impact assessments, and land use planning requires a comprehensive air quality assessment. This necessitates an understanding of the quantitative relationships among urban emissions, background air quality, ambient concentrations, atmospheric transport/dispersion/diffusion, photochemical reactions, and probable effects. To facilitate the design of strategic and effective research, pertinent research undertaken in Alberta was reviewed and synthesized in the context of national and international activities. Recommendations for future research were provided. The following research has been carried out to address two of the recommendations:

1. An urban air quality model(s) was selected for application in Alberta for use as a research tool and for airshed management;

2. The development and design of a detailed, comprehensive research and monitoring protocol to: (a) characterize and quantify temporal and spatial variability in the chemical and physical properties of urban aerosols, and (b) determine the relationship between visibility and particle mass loading.

## 4.2 ENVIRONMENTAL CONTAMINANTS

To assist in the planning and management of contaminants, short term research initiatives will address the identification of, movement of, dose effects of, evaluation of, and possible management strategies for contaminants. Over the longer term, research will investigate the impact and response of ecosystems to contaminant exposure and develop effective monitoring and control strategies.

Current research related to deposition is being carried out in two main areas:

### 4.2.1 Oil Sand Extraction and Discharge

The transport, transformation and removal, persistence and accumulation of contaminants from the heavy oil recovery and processing industry is being examined using the concept of fugacity.

### 4.2.2 Decommissioning

Research is directed at ensuring the proper abandonment of industrial and municipal facilities. A phosphogypsum study will identify the environmental and health hazards associated with phosphogypsum tailings. It will also assist in the development of national guidelines for disposal of phosphogypsum wastes.

## 5. EMISSIONS CONTROL TECHNOLOGY

Concurrent with the above studies, work will be undertaken on control technologies to reduce emissions at their source. For some time now, the Alberta Environmental Centre has been involved in the assessment and development of a wide range of concepts/processes for the control of acid-forming emissions. Source industries such as gas processing, thermal power, oil sands, gas compressors, and waste incineration have been considered. The use of magnesium oxide to remove sulphur dioxide gas from a sour gas processing plant has been developed and successfully tested at bench-scale. A pilot-scale project is under consideration for funding jointly by Alberta Environment and Environment Canada.

Assuming that feasible control technologies will some day eliminate emissions, and emission sources in some areas may be decommissioned over the intervening period, important questions will be: does the ecosystem recover on its own and, if so, how fast? Where possible, research will be focussed on these questions using field measurements in areas as the opportunities present themselves.

## 6. PROGRAM FRAMEWORK AND MANAGEMENT STRUCTURE

Overall co-ordination for the Departmental Acid Deposition Research Program is provided by the Research Management Division with the advice and support of the Departmental Committee on Acid Deposition (DCAD). Membership on DCAD includes representatives from Pollution Control Division (PCD), Standards and Approvals Division (SAD), Earth Sciences Division (ESD), Alberta Environmental Centre (AEC), Research Management Division, and other staff from the department to address specific program needs. DCAD addresses all activities, including research, monitoring, and ensures liaison between the Departmental, W&NC-LRTAP, and Government/Industry Acid Deposition Programs. The objective of the liaison is to share technical knowledge and prevent duplication of efforts. To ensure that comparable, scientifically valid methodology is used in the Departmental program, Alberta Environment has access to the Scientific Advisory Board of Government/Industry Acid Deposition Research Program.

A new system for approving research projects has been instituted by the Department. Program Planning Committees have been set up to identify and prioritize research projects to address major environmental issues in the province. In the case of acid deposition, the DCAD, as one of its functions, fills the requirement for an acid deposition research program planning committee. Where appropriate, Departmental project objectives and methodologies will be reviewed by external scientists and their suggestions incorporated.

## 7. CONCLUSION

The Acid Deposition, Air Quality, and Environmental Contaminants Programs for Alberta Environment should be viewed as a multi-faceted program of research, monitoring, and liaison activities which are designed to address all concerns for the potential effects of atmospheric deposition in the province. It is likely that research and monitoring activities will continue well into the 1990s, as long as concerns of Albertans regarding environmental and air quality persist.

## 8. ACKNOWLEDGEMENTS

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# Multimedia analysis and assessment of environmental toxic and carcinogenic substances: The regulatory connection.

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Abstract - Most toxic substance issues are multimedia in nature, but are managed within regulatory agencies as completely separate items. In New Jersey, the Office of Science & Research (OSR) has been involved in multimedia toxic substance assessments of air, water, food and soil contamination. These efforts have led to one of the best toxic substances inventories in the US, and perhaps the most far-reaching program of its kind at the state level. The research described in this report sets the foundation for development of an integrated toxic substances program. While volatile organic compounds (VOC) are used for illustration purposes, it is clear that multimedia analysis of human toxic substance exposures will be needed to develop cost-effective environmental solutions. To this end a new environmental health office is being initiated within OSR to address; risk assessment, risk reduction and risk communication aspects of a toxic substance regulatory program.

Introduction - The traditional segregation of pollution issues into water, air and solid wastes proved to be a workable arrangement, provided that the focus of each separate group was limited to media specific pollutants and/or pollutant measurements. Thus carbon monoxide is mostly an air pollution issue, while BOD or alkalinity are primarily water pollution issues. With the increasing emphasis on toxic and carcinogenic substances over the last 15 years, it has become clear that the traditional segregation of pollution issues does not allow for the intelligent development of sound regulatory programs.

In New Jersey (NJ), the Office of Science & Research (OSR) in the Department of Environmental Protection (DEP) has been conducting studies of toxic pollutants in ambient air, drinking water, industrial and sewage treatment plant effluents, contaminated fisheries, source emissions and other media (1-5). This information can be used to develop a unified approach for assessing toxic substances in NJ and for developing an overview for program integration within any environmental agency. To develop a comprehensive regulatory program for toxic substances accurate information is necessary in the following areas; pollutant sources, environmental levels, human exposures, health risks and mitigation methods. In addition, from our experience in NJ, one might consider a public communication program for all or some of these portions of a toxic substances regulatory program. Because OSR's primary focus has been on investigating toxic substances in the NJ environment, this paper will focus on pollutant sources, monitoring and human exposure efforts carried out in the state.

For the purpose of the present discussion, toxic and carcinogenic substances should be properly defined. In a review on airborne toxic pollutants, Goldstein (6) found it very difficult to develop an acceptable definition for these materials. In this paper, the definition of toxic and carcinogenic substances includes all those toxicants with the potential to cause human disease that are not currently subject to health based standards in the Clear Air Act of 1977 or the Safe Drinking Water Act of 1974. It should be emphasized that the development by US-EPA of the so-called priority pollutant list for water and the Pb standard in ambient air helped focus the scientific community's attention on toxic substances. The compounds provided in Table-1 are examples of toxic air pollutants and while this list identifies the major substances of concern, it is clear that we focus on a small percentage of the possible toxic substances in the air environment.

To reduce the discussion down to a manageable size, volatile organic compounds (VOC) will be the main subject for the remainder of this report. Volatile organic compounds can be defined as those organic substances with a vapor pressure greater than or equal to 0.02 psi at standard conditions. This class of pollutant includes; a) a number of human and/or animal carcinogens such as benzene or vinyl chloride (7), b) photochemically active precursors of smog components (8), c) common ground and surface water contaminants (9,10), and d) major components of industrial/commercial processes and consumer products (11,12).

Pollutant Sources - Information can be gathered on VOC sources utilizing both qualitative and quantitative approachs (13). Data on industrial/commercial usage of VOC is generally more available and of better reliability than emissions, effluent or waste disposal estimates. Likewise fuel usage information is a better qualitative indicator of losses to the environment than are estimates of emissions, leaking storage tanks, etc. For the toxic air pollutant projects carried out in NJ, Chemical and Engineering News Annual reports, the EPA's organic chemical producers data base, state air permit files, the industrial survey project (ISP) and fuel usage patterns were utilized to estimate the importance of various VOC sources to the air environment in the state (13,14). Currently, we are also conducting a limited number of combustion source emission tests for semi-volatile organic pollutants (SVOP) such as polycyclic aromatic hydrocarbons (PAH), nitro-PAH and PAH-quinones (15).

The NJ ISP provides a good example of how information on industrial emissions, discharges and waste disposal practices could be integrated into a comprehensive toxic substances program. The information on toluene shown in Figure-1 provides an indication of the distribution of industrial air emissions on a municipality basis in NJ. Also, industrial air emissions account for approximately 84% of the losses of this pollutant from this source category to the NJ environment. Obviously liquid fuel evaporative and combustion losses of toluene need to be factored into this assessment. With an understanding of the spatial distribution of VOC losses to the NJ environment, combined with environment fate and public health information, plausible hypotheses can be generated and investigated to fine tune assessing the significance of the various discharge vectors in a specific municipality. For example, it became clear from the ISP that significant quantities of VOC are discharged to publically owned treatment works (POTW's) in NJ. A subsequent investigation of a large, regional POTW in Middlesex County, indicated that these facilities are significant emissions sources for these materials (16). Considering that 75% of the industrial effluents in NJ are directed to POTW's (PC. Goldestein-NJPDES Program) and that VOC represent a significant portion of these discharges, serious attention by DEP is now being directed towards reducing VOC losses to the environment via this avenue. Such a pollutant reduction program would have multifaceted environmental benefits; a) a contribute to ozone control, b) reduce toxic substances levels in air, c) reduce the toxicity of POTW effluents and d) increase the options available for environmentally sound sewage sludge disposal.

It should be made clear that source information gathered from legally mandated surveys, such as statewide right-to-know programs, are inherently incomplete and not always accurate. Yet without information on pollution sources, a holistic toxic pollutant regulatory program cannot be developed.

Environmental Levels - Measurements of toxic pollutants are complex, costly and often imprecise (17, 19, 30). Concentration measurements in the environment provide an indication of; a) success of a regulatory program, b) influence of environmental fate on media-specific pollutant partitioning and c) can be used to approximate human exposures. Since toxic pollutants generally occur at low concentrations, large sample volumes, aggressive sample extraction, precise separation and specific/sensitive detection methods are necessary to carry out monitoring programs for environmental levels.

Various sampling strategies can be used to test or develop different hypotheses related to ambient concentrations of VOC and human health. In NJ, selected VOC have been monitored in the ambient air at urban and background sites, hazardous landfills, municipal landfills, industrial sites and at a POTW (16,21,24). These different studies help provide a prospective for where the greatest regulatory effort should be directed to minimize human exposure to these toxic substances via the air route. The information provided in Table-2 for four selected VOC indicates that localized, source specific problems need to be addressed prior to efforts with area sources. However, it was also discovered that small, localized VOC sources may be more important than large sources. For example during the NJ program on Airborne Toxic Elements and Organic Substances (ATEOS), a localized truck body manufacturer was identified as a major contributor to the peak toluene levels found at the Newark site (Figure -2), but not at the other two locales studied (25).

Investigations of potable water supplies in NJ indicate that certain VOC are found in both ground and surface waters (2, 10, 26). The most commonly found substances in groundwater, such as trichloroethylene or methylene chloride, are somewhat polar and are high use solvents. Significant localized groundwater degradation by VOC has been documented in NJ due to improper waste disposal practices, spills, leaking underground storage tanks and/or chemicals associated with septage tanks (9, 10, 27). Some trihalomethanes appear to be frequently found in certain finished potable water supplies subject to chlorination (26).

Information on food and indoor air pollution VOC levels have not been extensively documented in NJ. However, the initial results from the studies of personal exposures to selected VOC in Elizabeth and Bayonne, NJ (TEAM study) indicate that indoor levels may be significantly elevated over outdoor levels (28). Outside of serious concerns about the quality of the reported measurements, any subsequent verification of the TEAM study results poses an interesting dilemna for the regulatory community (29). At the very least regulatory programs will need to be coordinated between consumer product, occupational health and environmental protection agencies to achieve the greatest exposure reduction in the most cost effective manner. If selected VOC are associated with food precessing and packaging then perhaps this vector of exposure needs to be better documented. Limited information has been developed for VOC in food, thus substances such as ethylene dibromide, methylene chloride, chloroform and styrene have been found in food supplies (30).

Human exposures - The state-of-the-art for exposure assessment related to toxic substances has advanced significantly in the last 10 years (31). Different levels of assumptions are needed depending on the exposure assessment approach adopted by a regulatory agency. Recently, EPA adopted guidelines for conducting exposure assessments (32), while specific attention has been directed towards this issue in air pollution epidemiology (33). Modelling approachs for air, water and/or waste disposal scenarios which combine information on environmental fate provide the crudest estimates of human exposures. Such an approach was utilized for selected atmospheric toxic substances by EPA in the so-called Six Month Study (34).

The next level of sophistication in exposure assessment comes from combining environmental monitoring data, with time/activity patterns and assumptions on human physiological and internal partitioning factors. Such an effort has been carried out with perchloroethylene data collected during the ATEOS project (35). Utilizing the standard approach described by CAG (36), lung absorption patterns for perchloroethylene (37), time/activity patterns (38), and extrapolation from indoor/outdoor ratios of $SO_2$ (39), the exposure estimates are provided for three NJ locales (Table-3). While Wallace et al (28) found indoor/outdoor ratios for perchloroethylene of 2.2 from data collected in NJ homes, because of data quality issues associated with this study, we view the TEAM results with a measure of skepticism. Thus until the TEAM results are verified, we prefer to assume that perchloroethylene is primarily on outdoor pollutant. Burke (26) compared selected VOC exposures for individuals in NJ on the basis of ground or surface water use and atmospheric concentrations (Table 4-5). Although the assumptions made in the study cannot be completely justified, it is clear from this analysis that the air route of exposure is more often than not the greatest source of VOC exposures in NJ. From such an analysis, regulatory agencies could begin to develop more focused and cost effective pollution control programs in their respective jurisdictions.

Carrying our personal monitoring provides the most appropriate estimate of exposure based on our current understanding of this issue (40). Most personal monitoring for environmental contaminants have been based on single exposure vector pollutants (air), such as carbon monoxide or respirable particles (31). The information collected in NJ during the TEAM study included water samples (41) and thus this study added an additional level of sophistication to the exposure assessment field. Most toxic substances, including VOC, are found in air, water and food. Accurate personal monitoring studies must include the measurement of all three exposures vectors. This is particularly true since many toxic substances are either bioconcentrated in foods or are efficiently adsorbed by foods during processing, packaging, storage and preparation. A total human exposure study (THES) of benzo(a)pyrene (BaP) has been initiated in NJ. During the THES project, personal air, water, food and soil levels of BaP will be documented to develop a complete exposure picture for this carcinogenic pollutant (42). To the best of our knowledge, this modest effort represents the first attempt to address all major exposure routes for a toxic substance.

A new and very promising area for accurate exposure assessments is chemical and biochemical dosimetry (43). Harris (44) provides a good overview of the advantages and caveats associated with the utilization of DNA-adduct dosimetry. In essence this approach reduces all the exposure routes, environmental fate and human detoxification processes down to a single measure of exposure. While the formation of DNA-adducts appears to be an important step in the development of certain cancers, additional studies are necessary to evaluate whether DNA-adducts are good estimates of exposure and are accurate qualitative and/or quantitative indicators of disease (44). In the NJ THES project, we have proposed working with researchers at a major medical school to assess BaP-DNA-adducts in the blood of our test population (45). Although the THES project is limited in scope, the measurement of BaP-DNA-adducts and with corresponding air, water, food and soil exposure estimates, may provide the most tightly linked environmental toxic substance exposure study conducted to date.

Synthesis - The toxic substances program in OSR has led to the development and evolution of many key regulatory programs in NJ. Examples of some programs developed from OSR's efforts include those associated with hazardous site cleanup, drinking water, groundwater, toxic air pollutants and contaminated fisheries. As the questions being asked of our regulatory agency become more complex, these efforts need to become better integrated. A new health assessment program has been initiated in OSR and this program will have three major components: risk assessment, risk reduction and risk communication. In short, what is intended is to combine our environmental toxic substance investigation abilities with state-of-the-art health assessment, pollution abatement and public communication/participation programs, so that the DEP has the best possible tools to make sound regulatory decisions in the years ahead. This program is a great challenge; finding environmental problems is relatively easy, developing and implementing state-of-the-art solutions is much more difficult. Combining the best science, engineering and social science information will hopefully lead to cost-effective reductions in environment exposures to toxic substances in NJ.

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## TABLE 1

### Examples of Toxic Air Pollutants

| Particles | | Gases |
|---|---|---|
| Organic | Inorganic | |
| PAH | soot | halogenated hydrocarbons |
| Pesticides | asbestos | aromatics |
| PCB's | silicates | aldehydes |
| TCDD | trace metals | ketones |
| phthalates | radionuclides | nitrosamines |
| carboxylic acids | sulfate | epoxides |
| thiophenes | nitrate | ethers |
| aromatic amines | | lactones |
| epoxides | | nitroalkanes |

From Harkov (7)

TABLE 2

Comparison of Mean Ambient Concentrations of Four Selected VOC at Various Monitoring Locales in New Jersey (ppbv)

| | Site Description | | | | |
|---|---|---|---|---|---|
| Pollutant | Baststo[46] Rural Site | Newark[21] Urban/ Industrial Site | Edgeboro[22] MSW Landfill | Kramer[22] Superfund Site | MCUA[16] Grit/Screen Area-POTW |
| Benzene | 0.61 | 2.61 | 3.33 | 7.51 | 9.9 |
| Toluene | 0.59 | 4.93 | 27.8 | 46.5 | 88.7 |
| Trichlor-oethylene | 0.08 | 0.59 | 0.32 | 2.43 | 2.4 |
| perchloro-ethylene | 0.04 | 0.46 | 1.42 | 1.91 | 3.9 |
| N= | 42 | 39 | 15 | 15 | 15 |

## TABLE 3

## Exposure Estimates for Perchloroethylene from the ATEOS Program

| Communities | Annual (mg/kg/day) |
|---|---|
| Camden | $0.178 \times 10^{-4}$ |
| Newark | $0.280 \times 10^{-4}$ |
| Elizabeth | $0.262 \times 10^{-4}$ |

From Harkov (35)

## TABLE 4

### Comparison of Daily Exposure Levels of Six Common Pollutants in Drinking Water and Air

| | Geo Mean Concentration | | Daily Exposure[(a)] | | % of Total Exposure | |
|---|---|---|---|---|---|---|
| **Compound** | **Air** | **Water** | **Air** | **Water** | **Air** | **Water** |
| | | | **Surface Water Consumers** | | | |
| Methylene chloride | 2.30 | 2.34 | 46.00 | 4.68 | 91% | 9% |
| Chloroform | .63 | 21.47 | 12.60 | 42.94 | 23 | 77 |
| Trichloroethylene | 2.16 | 1.91 | 43.20 | 3.82 | 92 | 8 |
| Tetrachloroethylene | 2.49 | .04 | 49.80 | .08 | 100 | 0 |
| Benzene | 5.62 | 5.73 | 112.40 | 11.46 | 91 | 9 |
| Toluene | 12.65 | 16.45 | 253.00 | 32.90 | 88 | 12 |
| Total | | | 517.00 | 95.88 | 84 | 16 |
| | | | **Ground Water Consumers** | | | |
| Methylene chloride | 2.30 | 27.32 | 6.00 | 54.64 | 46 | 54 |
| Chloroform | .63 | 3.48 | 12.60 | 6.96 | 64 | 36 |
| Trichloroethylene | 2.16 | 9.61 | 43.20 | 19.22 | 69 | 31 |
| Tetrachloroethylene | 2.49 | 4.48 | 49.80 | 8.96 | 85 | 15 |
| Benzene | 5.62 | 4.45 | 112.40 | 8.90 | 93 | 7 |
| Toluene | 12.65 | 7.72 | 253.00 | 15.44 | 94 | 6 |
| Total | | | 517.00 | 114.12 | 82 | 18 |

[(a)] Assumes daily consumption of 2 liters of water and 20 cubic meters of air.

Note: Air measured in ug/cubic meter; water in ug/liter.

From Burke (26)

## TABLE 5

## Comparison of Daily Exposure Levels from Drinking Water and Air in Three New Jersey Cities

| | Geo Mean Concentration | | Daily Exposure[a] | | % of Total Exposure | |
|---|---|---|---|---|---|---|
| Compound | Air | Water | Air | Water | Air | |
| | | | Camden | | | |
| Methylene chloride | 3.50 | 2233.00 | 20.00 | 4466.0 | 2% | 98% |
| Chloroform | .61 | .90 | 12.20 | 1.8 | 87 | 13 |
| Trichloroethylene | 1.45 | 14.25 | 29.00 | 28.5 | 50 | 50 |
| Tetrachloroethylene | 1.87 | 9.10 | 37.40 | 18.2 | 67 | 33 |
| Benzene | 5.57 | 0.00 | 111.40 | 0.0 | 100 | 0 |
| Toluene | 9.41 | 0.00 | 188.20 | 0.0 | 100 | 0 |
| Total | | | 448.20 | 4514.5 | 9 | 91 |
| | | | Elizabeth | | | |
| Methylene chloride | 1.72 | 20.55 | 34.4 | 41.10 | 46 | 54 |
| Chloroform | .53 | 62.93 | 10.6 | 125.86 | 8 | 92 |
| Trichloroethylene | 3.08 | 0.00 | 61.6 | 0.00 | 100 | 0 |
| Tetrachloroethylene | 2.95 | 0.00 | 59.0 | 0.00 | 100 | 0 |
| Benzene | 5.14 | 0.00 | 102.8 | 0.00 | 100 | 0 |
| Toluene | 16.63 | 0.00 | 332.6 | 0.00 | 100 | 0 |
| Total | | | 601.0 | 166.96 | 78 | 22 |
| | | | Newark | | | |
| Methylene chloride | 2.03 | 0.00 | 40.6 | 0.0 | 100 | 0 |
| Chloroform | .87 | 40.95 | 17.4 | 81.9 | 18 | 82 |
| Trichloroethylene | 2.24 | 0.00 | 44.8 | 0.0 | 100 | 0 |
| Tetrachloroethylene | 2.81 | 0.00 | 56.2 | 0.0 | 100 | 0 |
| Benzene | 6.20 | 9.00 | 124.0 | 18.0 | 87 | 13 |
| Toluene | 12.93 | 67.45 | 258.6 | 134.9 | 66 | 34 |
| Total | | | 541.6 | 234.8 | 70 | 30 |

(a) Assumes daily consumption of 2 liters of water and 20 cubic meters of air.

Note: Air is measured in ug/cubic meter; water is measured in ug/liter.

From Burke (26)

Figure 1 Municipality based toluene air releases from industrial sources in New Jersey (Steveson et al 15).

Figure 2 Daily toluene variations at four New Jersey sites during the summer of 1981 (Harkov et al 25).

# TOLUENE

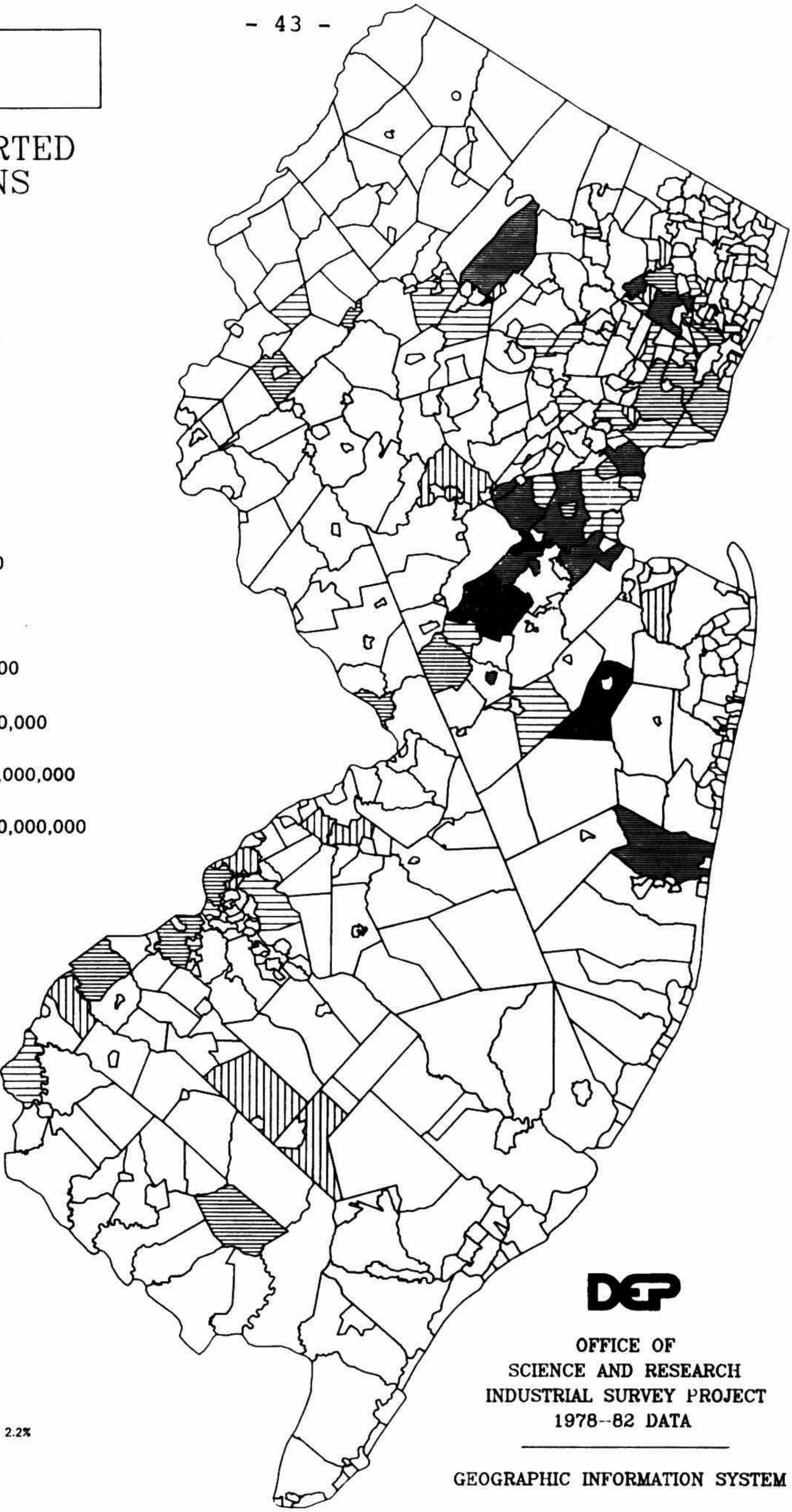
## TOTAL REPORTED AIR EMISSIONS

### BY MUNICIPALITY

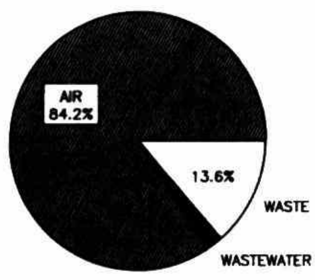
*This map illustrates levels of the chemical reported in one environmental parameter. It is not intended to show exposure levels.*

## POUNDS

- ☐ NONE REPORTED
- ▥ LESS THAN 1,000
- ▤ 1,000 TO 10,000
- ▤ 10,000 TO 100,000
- ▩ 100,000 TO 1,000,000
- ■ 1,000,000 TO 10,000,000
- ■ 10,000,000 TO 50,000,000



TOLUENE ENVIRONMENTAL RELEASES



TOTAL RELEASE = 36703000 POUNDS

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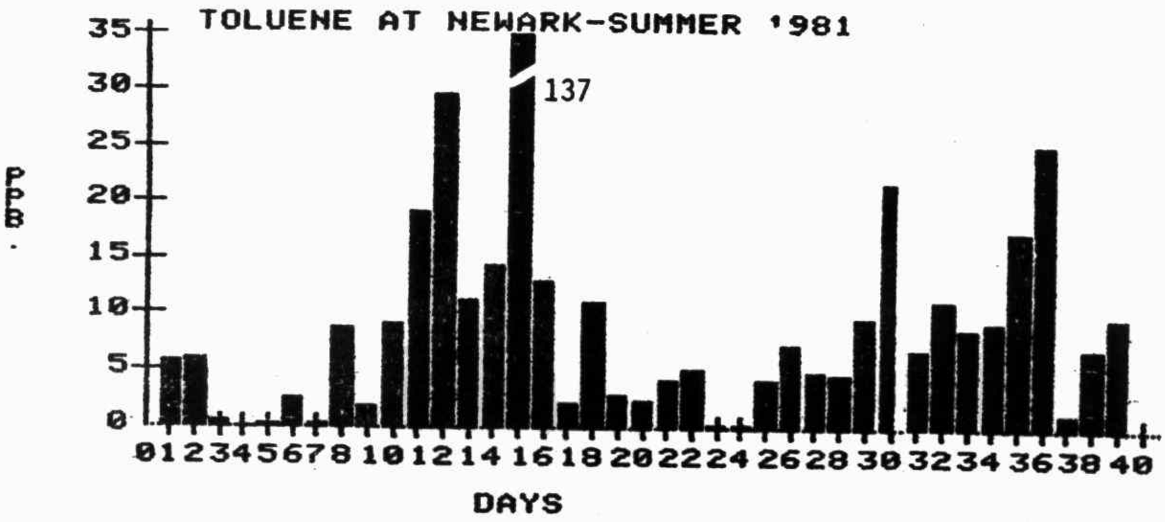
1978--82 DATA

GEOGRAPHIC INFORMATION SYSTEM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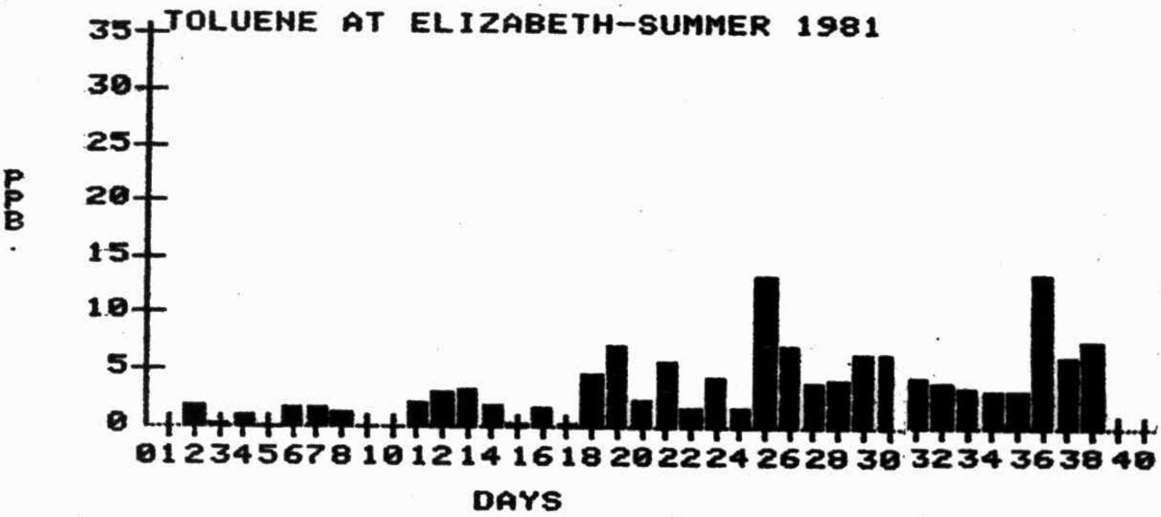
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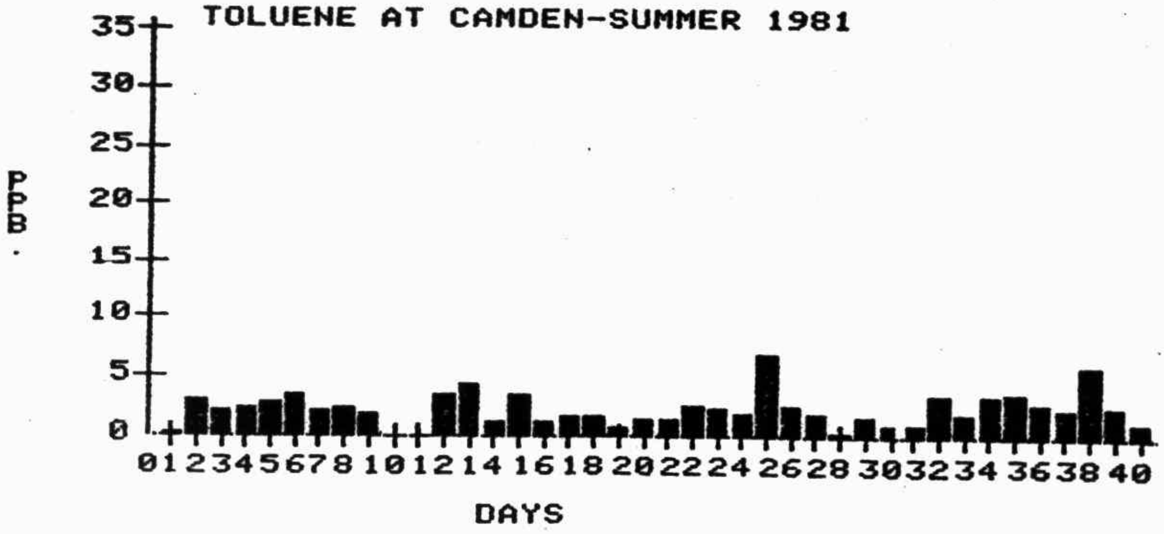
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# THE DEVELOPMENT OF TECHNIQUES FOR THE RAPID SCREENING OF MUNICIPAL REFUSE INCINERATOR FLY-ASH AND STACK GAS FOR PCDD/PCDF USING A TRANSPORTABLE TANDEM MASS SPECTROMETER (GC/MS/MS) SYSTEM

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## 1.0 FLY-ASH

### 1.1 INTRODUCTION

The incineration of municipal refuse for the purposes of energy recovery has been linked to the production of undesirable byproducts notably polychlorinated-dioxins and -dibenzofurans (PCDD/PCDF). Since these substances are found in incinerator stack gases and fly-ash in miniscule amounts, methods for the analysis of these trace chemicals must therefore be sensitive and selective. This is currently accomplished by time and resource consuming wet chemical clean-up steps followed by gas chromatography mass spectrometric (GC/MS) analysis. The goal of obviating the time and resource consuming clean-up stages by using more specific analytical methods is a desirable one, since much of the cost for such sample analyses is in these clean-up stages. Furthermore, if analyses could be performed more cheaply and faster, more information could be gained which would lead to a better understanding of the processes involved in the formation of substances like PCDD/PCDF during incineration.

The goals of the present work were to investigate GC/MS/MS analysis of rapidly derived extracts of municipal refuse incinerator fly-ash. Tandem mass spectrometry (MS/MS) has demonstrated high specificity for the analysis of trace substances and, since its introduction of the world's first commercially available tandem triple quadrupole MS/MS system, SCIEX® has been involved in investigating novel ways of applying this new technology to solve problems in trace chemical analysis more rapidly. This type of development work has been carried out by SCIEX® in the past for the analysis of tetrachlorodibenzodioxin (TCDD) in soils under contract with the US-EPA (Region VII) and for the detection of polychlorinated biphenyls (PCBs) in stack gases under contract with MOE for example.

The scope of work performed by SCIEX® in this study was to:

(i) Develop a rapid "jar"-extraction* method for municipal refuse incinerator fly-ash which: (a) has the highest possible relative extraction efficiencies, (b) requires minimum sample handling, and (c) is amenable to GC/MS/MS analysis;

(ii) Determine whether crude (i.e. rapidly derived) fly-ash extracts exhibit interferences during GC/MS/MS analysis;

(iii) Compare "jar"-extraction efficiencies with those of conventional (Soxhlet) extraction methods using a variety of fly-ash types; and

(iv) Determine the reproducibility of the procedure.

## 1.2 EXPERIMENTAL

Three types of fly-ash samples were used in this development program: types C (Commissioner Street), A (Ashbridge Bay) and S (SWARU). Jar-extract method development was carried out using a sample of well characterised fly-ash of type C supplied to SCIEX® by MOE-LSB. This was used to ascertain which solvent systems supplied the best relative extraction efficiencies. The absolute extraction efficiencies would be tested by comparing jar-extracts to extracts derived by conventional 18h Soxhlet extraction methods. The conventional extracts were supplied by MOE-LSB and the other contract laboratory (Wellington Environmental Consultants (WEC)) involved in this study.

An extracting solvent comprising 2 parts toluene and 1 part methanol was found to provide relatively good extraction efficiencies for the majority of PCDD/PCDF congeners. A flow chart for the jar-extraction procedure can be found in last year's proceedings of this conference.

The tandem mass spectrometer used in the present work was the TAGA® 6000E tandem triple quadrupole MS/MS system manufactured by SCIEX®. A GC is directly coupled to the MS/MS system's ion source for sample introduction. The ion source is a Glow Discharge ion source at 200°C which uses Zero Air as the reagent gas at 35 ml/min flow providing a source pressure of some 1 Torr. The $N_2/O_2$ reagent gas mixture provides an efficient charge transfer medium for the ionization of aromatic hydrocarbons such as PCDD/PCDF while the oxygen acts in a cleansing fashion keeping ion source parts clean by "burning" off carbon deposits. Thus, crude extracts can be analyzed without having to clean the ion source after use. The discharge current was 1.2 mA and the molecular ions created in the source and selected by Quadrupole 1 (the first mass spectrometer) were fragmented in the quadrupole collision cell (Quad 2) by 65 eV collisions with Argon at $4 \times 14^{14}$ atoms/$cm^2$ target gas thickness (see Schematic Figure 1). Daughter ions, specific to PCDD/PCDF are mass selected in Quadrupole 3 and detected by pulse counting. The GC conditions and a summary of GC/MS/MS analytical conditions can be found in last year's proceedings. The $^{13}C$-PCDD/PCDF labelled components were added in the jar-extraction procedure in known amounts and act as internal standards for quantitation purposes. GC/MS/MS data were acquired 6.5 minutes into the GC run after injection of the crude jar extract. There were 7 groups of Q1/Q3 parent ion/daughter ion pairs covering the whole range of PCDD/PCDF congeners and the isotopically labelled internal standards. The quadrupole mass spectrometer settings and all other instrumental parameters are under computer control. The times for switching ion sets were determined by injection of a previously analysed fly-ash extract. This type of GC/MS/MS analytical procedure is termed multiple reaction monitoring (MRM) since the instrument is detecting the products of collisionally activated decomposition (CAD) ion-molecular reactions occurring between the mass resolving quadrupoles. The detection limits for the GC/MS/MS system were found to be in the sub picogram (pg) to 3 pg range for the injection of PCDD/PCDF standards.

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* The term "jar"-extraction means simply a method of extraction requiring only the mixing of the matrix (fly-ash) with a suitable extracting solvent and mixing prior to analysis.

## 1.3 RESULTS AND DISCUSSION

The jar-extraction efficiencies were compared to conventional extraction methods for fly-ash sample C1 and the result is shown in Figures 2 and 3. The relative extraction efficiency was reasonably good for the lower chlorinated congeners but dropped off rapidly as the degree of chlorination increased.

The reproducibility of the techniques was demonstrated by analysing three C4 jar-extracts. The results are summarized in Figures 4 and 5 and demonstrate an average standard deviation of about ±10%.

In addition to the test of analytical reproducibility, QA/QC samples, comprised of crude and cleaned-up extracts of C1 and S1 fly-ash samples (derived by conventional extraction methods and furnished by MOE-LSB and WEC respectively) were analysed by GC/MS/MS and the results compared (see last year's proceedings and Figures 6 and 7).

The levels of PCDD/PCDF between crude and cleaned-up conventional fly-ash extracts shown in these figures indicate that both the other participating laboratories (MOE-LSB and WEC) produced extracts comprising comparable levels of PCDD/PCDF even though their degree of clean-up was substantially different, i.e. as per our QC/QC protocol MOE-LSB supplied a crude extract (no clean-up) while WEC supplied an extract which was taken through an extensive clean-up procedure. The fact that the GC/MS/MS results showed comparable levels for both crude and cleaned-up extracts indicates that the GC/MS/MS analytical technique is unperturbed by the other chemical constituents which make up the bulk of the fly-ash matrix. Furthermore, the results obtained by GC/MS/MS compared favourably with those obtained by GC/MS on the cleaned-up extract, establishing equivalency between the two analytical techniques.

## 1.4 CONCLUSIONS

The jar-extraction procedure followed by GC/MS/MS analysis is a rapid technique for the determination of PCDD/PCDF in fly-ash requiring less than 2 hours per sample. Although the reproducibility is within reasonable limits ($\pm$ 9-11%) the extraction efficiencies were lower than those of conventional methods. The extraction efficiencies were found to be inversely proportional to the degree of chlorination and ranged from 75% for dichloro- congeners to 24% for octachloro- congeners. Not all fly-ash samples were amenable to the jar-extraction procedure. Type S fly-ash exhibited much lower extraction efficiencies and was, upon visual inspection, higher in carbon content. The crude C- and S-type extracts derived by conventional extraction exhibited no significant interferences when run by GC/MS/MS which bodes well for eliminating clean-up steps. Furthermore, the fact that the TAGA® 6000E MS/MS system is capable of transport within a mobile laboratory makes the jar-extraction GC/MS/MS analytical scenario amenable to on-site determinations of PCDD/PCDF at their source of production.

## 2.0 STACK GAS

### 2.1 INTRODUCTION

This portion of the paper is a brief account of the development of procedures for analysis of modified MM-5 stack gas sampling trains for quantities of PCDD/PCDF using GC/MS/MS. For this study a parallel analysis of the same stack gas using unmodified MM-5 sampling trains and GC/MS was undertaken. The goals of this section were to: (1) determine whether quantities of PCDD/PCDF are sufficiently high in municipal incinerator stack gas to permit a rapid GC/MS/MS analysis of crude XAD-2 resin extracts; (2) ascertain which fraction of total PCDD/PCDF is transmitted through the sampling train to the XAD-2 resin; and (3) assess whether the XAD-2 resin or the organics in the stack gas pose matrix problems for the GC/MS/MS analytical procedure when the XAD-2 resin is jar-extracted and the extract analyzed without cleanup stages.

The ultimate goal of the present work is to determine if the methods explored in this study can be practically implemented at the incinerator site in a mobile laboratory, outfitted with a transportable GC/MS/MS analytical system and rudimentary wet-laboratory facility. Accordingly, an attempt has been made to keep all sample handling and work-up methods as simple and as rapid as possible.

Stack sampling was done using parallel trains (modified MM-5 [see below] and unmodified MM-5) using XAD-2 as the sorbent resin to trap organics, including PCDD/PCDF. A smaller sorbent cartridge - one which contained 20 g of XAD-2 resin instead of the 100 g normally used - was the only difference between the modified and unmodified MM-5 trains. A schematic of the modified stack gas sampling "cartridge" is shown in Figure 8. About 2m$^3$ of stack gas were drawn through both trains' cartridges after passing through heated probe (P), particulate filter (PF), and filter bottom and connector (FBC). The modified MM-5 train cartridge contained two 10 g portions of XAD-2 resin separated by a glass wool partition. In both trains, stack gas and condensed water flow through the XAD resin and organics are adsorbed onto the sorbent material. In the modified cartridge the top 10 g resin portion adsorbs the organics and the bottom portion is used to recover any breakthrough.

The stacks were sampled isokinetically for approximately 180 min/run and sampling line temperatures were maintained at 125°C. The ends of the two parallel sampling probes were situated close to each other in the stack, and an equal volume (ca. 2 $m^3$) of stack gas drawn through each of the respective trains. Two incinerators were tested: a municipal refuse incinerator (MRI) (Commissioner Street) and a sewage sludge incinerator (SSI) (Ashbridge Bay). The MRI had a stack temperature of ca. 225°C and a flow of ca. 78 $Nm^3$/sec, and the SSI, a stack gas temperature of ca. 35°C and a flow of ca. 36 $Nm^3$/sec.

Prior to the GC/MS/MS analysis, the upper XAD-2 resin portion was subjected to the following analytical procedure:

1) Water was removed from the cartridge by first flushing with methanol, followed by purging with UHP Nitrogen at room temperature.

2) The top XAD-2 portion was emptied into a clean 50 ml centrifuge tube (see Figure 9) and spiked with 20 ul of a solution of ISI-200, the stock solution of isotopically labelled internal standards.

3) 4 X 5 ml aliquots of toluene were poured through the empty glass condenser/cartridge and collected in a graduated cylinder. The level of toluene in the cylinder was brought to 20.0 ml and transfered to the centrifuge tube containing the XAD-2 resin.

4) The centrifuge tube containing XAD resin and solvent was then vortexed (30 sec), and placed in an ultrasonic bath for ca. 0.5 hours.

5) A glass tube with a frit near the bottom was inserted into the centrifuge tube (see Figure 9) and the mixture centrifuged for 10 minutes forcing toluene through the frit and up the tube.

6) A 5 ml aliquot of toluene was then removed from the glass tube, and blown down to dryness and taken up with 5 ul solvent (toluene).

7) Depending on the levels of matrix and PCDD/PCDF, these solutions were diluted before being analyzed directly by GC/MS/MS.

All method development for the above "jar"-extraction procedure was carried out using the first two MRI samples. Altogether there were 5

MRI and 4 SSI samples run by GC/MS/MS and GC/MS. After stack sampling, the probe, and filter bottom connector from the unmodified MM-5 train were rinsed with solvent and analyzed by GC/MS after multi-column clean-up steps. The XAD-2 resin was extracted and analyzed by GC/MS using standard protocols[1] incorporating multi-column clean-up steps and isotopically labelled internal standards for quantitation. The GC/MS analysis results comprised two sets of data: quantities of PCDD/PCDF in (1) the probe and filter container; and (2) the condensor and XAD-2 resin. All GC/MS analyses were performed on cleaned-up extracts while GC/MS/MS analyses were performed on the rapidly derived crude jar extracts.

Prior to analysis of the modified MM-5 train samples by GC/MS/MS, a jar-extraction method development using clean XAD-2 resin indicated quantitative removal of test compounds OCDD and 1,2,3,4-TCDD from the resin employing MeOH/toluene mixtures which were as low as 20% in toluene. It was discovered during the analysis of the first stack gas sample that once XAD-2 resin is exposed to $2m^3$ of stack gas and condensate, it does not behave like the original material. In fact, it was necessary to use 100% toluene to ensure quantitative extraction of PCDD/PCDF from the used XAD-2. Treatment of the XAD-2 resin with MeOH while still in the cartridge provided an effective way of removing residual water prior to toluene extraction. When drawn through the cartridge by suction, methanol does not disturb the organic matter adsorbed onto the XAD-2 resins.

It was suspected that the analysis of crude XAD-2 extracts may demonstrate large mass spectral interferences due to the high organic content of the stack gas and that of the degraded XAD-2 resin after treatment with stack gas condensate. It is clear from Figure 10 that MS/MS provides sufficient specificity to overcome mass spectral interferences arising from the chemical matrix for tetrachloro- congeners. The two chromatograms shown in this figure are those of TCDD congeners analyzed by GC/MS/MS (m/z 320 fragmenting to m/z 259). The top chromatogram is that of a jar extract of fly-ash and the bottom that of a jar extract of XAD-2 resin, each run without clean-up. The isomer distribution for the two samples are almost identifical indicating that neither the XAD-2 resin, nor the extraneous organic matter in the stack gas, interferes in the MS/MS detection of TCDD. It is interesting to note that each of these samples came from the same incinerator except that the fly-ash was obtained and analyzed several months prior to the stack gas sample.

Unfortunately, one source of matrix interference not related to the tandem mass spectrometer analyzer was encountered for some MRI and all SSI stack gas samples. This non-mass spectral interference was due to a titration of the ionizing capacity of the chemical ionization ion source by the chemical matrix. The very large amount of neutrals coming from the GC column and passing through the ionization region is responsible for this effect. Such an interference leads to a complete suppression of response where even the isotopically labelled internal standards added to the crude extract are unobserved. Such an unfortunate result is understandable since the XAD-2 resin was observed to be very brown and discoloured with organic contamination after sampling. Furthermore, concentrated jar extracts of this resin looked more like a crude oily sludge than a solvent extract. The jar extracts from SSI stack gas appeared even dirtier than those from the MRI.

## 2.2 RESULTS & DISCUSSION

The levels of PCDD/PCDF from the MRI were enough to obtain good signal-to-noise for both GC/MS and GC/MS/MS analysis protocols. Figure 11 demonstrates the levels of PCDD and PCDF broken down into congener groups for the MRI samples. The average total PCDF and PCDD levels emanating from stack were 7,303 ng/m$^3$ (or 2 gm/hr) and 867 ng/m$^3$ (or 0.25 gm/hr) respectively. The highest levels were in the tetra- and penta- chlorinated congener groups. Although the mechanisms of dioxin and furan formation in the MRI are not well understood, the fact that PCDF are at higher levels than PCDD suggests that chlorinated dibenzofuran precursor's (e.g. PCBs) are higher in the feed stock than are chlorinated dioxin precursors (e.g. chlorinated phenols). Figure 12 is that of PCDD/PCDF levels found in SSI stack gas. Immediately one can see that PCDD/PCDF levels are considerably lower in SSI than in MRI stack gas especially in the tetra- and penta- chlorinated congener groups. The average levels observed are 1,265 ng/m$^3$ (or 0.2 gm/hr) for PCDD and 235 ng/m$^3$ (or 0.03 gm/hr) for PCDF, where the highest levels are those for the lower chlorinated congeners, especially dichlorodibenzo-p-dioxins ($D_2CDD$). This propensity toward lower chlorinated species in SSI stack gas may be a function of the lower stack gas temperatures where higher chlorinated constituents are precipitated out in scrubbing or cooling stages prior to entering the stack.

When we looked more closely at the distribution of PCDD/PCDF throughout the sampling train it was discovered that a good fraction was found in areas other than the XAD-2 cartridge. Figure 13 shows the partitioning of PCDF between the probe portion of the train and the XAD-2 condenser/ trap portion (so called "Probe" and "Trap" respectively). It is apparent that most of the PCDF is found in the trap, however, a significant amount is in the probe portion of the train. For the PCDD there are almost equal amounts in the probe and trap portions of the train (see Figure 14). Furthermore, under more careful scrutiny, the inter-congener and inter-run partitioning varies considerably (see Figure 15). There is, however, a general trend; i.e. with the exception of one of the runs, when going to higher chlorinated congeners, there are proportionally more PCDD/ PCDF found in the probe than in the trap. Thus, for tetrachlorinated congeners, there is 2 to 5 times the amount of $T_4CDD$ and $T_4CDF$ in the Trap than in the Probe, whereas for octachlorinated species, there is anywhere from 2 to 6 times the amount of material in the Probe than in the Trap.

This result is rather disappointing since, when we proposed this research, it was hoped that most of the vapour phase PCDD/PCDF would have made it through the sampling train to the XAD-2 cartridge. This, more than any other finding, leads to serious doubts that a rapid method of accurately determining PCDD/PCDF in municipal refuse incinerator stack gas can be accomplished using any analytical technique, as long as the transport of PCDD/PCDF through the sampling train shows these variable and low transport efficiencies.

In all cases, the MS/MS detector showed little interference to the XAD-2 and stack gas matrix however the shear amount of organic matter in these crude extracts had a deleterious effect on the ionizing capacity of the ion source in some of the samples. Because of this, no quantitative data could be rendered for two of the MRI samples or any of the SSI samples by GC/MS/MS of the jar-extracts. Where quantitative data were obtained for the MRI samples, there was little good correlation between the results using the modified MM-5 train and GC/MS/MS and the unmodified MM-5 train and GC/MS. This is likely due to the variable and non-quantitative properties of the sampling trains toward PCDD/PCDF. Perhaps maintaining the train temperatures higher than 125°C would permit better transmission of PCDD/PCDF to the XAD-2 resin cartridge. Results for PCDD/PCDF analysis by GC/MS and GC/MS/MS of respective XAD-2 resin samples for one of the runs did show a moderately good correlation (see Figure 16) however, this was more the exception than the rule and certainly, with the variability in PCDD/PCDF transmission, the usefulness of such a correlation is questionable.

With respect to the SSI samples, significant amounts of DCDD (and DCDF, $T_3$CDD and $T_3$CDF for MRI) were easily detectible by GC/MS/MS, however, we were unable to quantitate these constituents since the response to the isotopically labelled internal standard ($^{13}$C-TCDD/TCDF) was negligible due to the aforementioned matrix suppression. The DCDD was apparently unaffected by this matrix since it elutes prior to the suppression components. Because the sample matrix is so unpredictable, all quantitation of native compounds must be undertaken by ratioing signals due to native PCDD/PCDF with those of isotopically-labelled internal standards. Quantitation using external standards is highly speculative using either GC/MS/MS or GC/MS. The GC/MS analysis of the cleaned up extracts had no problem quantitating these analytes due to the elimination of the ion source suppressing matrix constituents.

## 2.3 CONCLUSIONS

1) There are sufficient quantities of PCDD/PCDF in municipal-refuse incinerator stack gas to be detected using the above sampling protocols.

2) The stack sampling apparatus was inadequate for the quantitative (i.e. 100%) transmission of vapour phase PCDD/PCDF to the XAD-2 resin cartridge. Better heat tracing must be used on the stack sampling train to increase transmission to the XAD cartridge.

3) MS/MS analysis demonstrated freedom from interferences from isobaric compounds.

4) As a final QA/QC protocol, a cleaned up sample(s) already run by GC/MS, should be analyzed by GC/MS/MS.

5) Many of the crude XAD-2 resin extracts were sufficiently contaminated with large amounts of organic matter to suppress ion-source chemical ionization capabilities as well as to irreversibly damage several capillary GC columns.

6) More research should be undertaken to mitigate the effects of chemical matrix on GC/MS/MS analysis stressing the development of rapid cleanup procedures.

The results of this phase of the FLY-ASH and STACK GAS research programs are positive enough to represent a significant increase in analytical capability in the area of PCDD/PCDF analysis. The cooperation between the MOE and SCIEX® has contributed substantially to the applications' development of tandem triple quadrupole GC/MS/MS technology, an example of high-technology analytical equipment manufactured in Ontario. This research has benefitted SCIEX® by expanding the application base of its technology as well as providing a more rapid and cheaper method of determining dangerous environmental contaminants such as PCDD/PCDF.

## 2.4 REFERENCE

1. L. L. Lamparsky, T. J. Nestrick, and R. H. Stehl, Anal. Chem. 51, 1453, 1985.

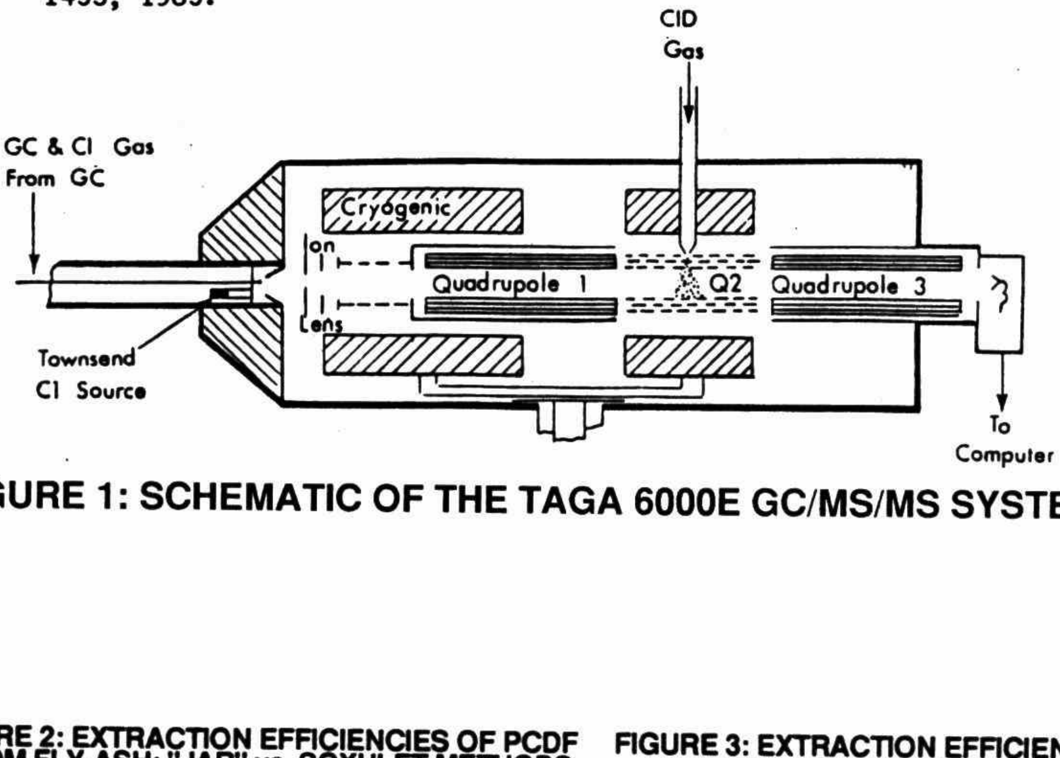


FIGURE 1: SCHEMATIC OF THE TAGA 6000E GC/MS/MS SYSTEM

FIGURE 2: EXTRACTION EFFICIENCIES OF PCDF FROM FLY-ASH: "JAR" vs. SOXHLET METHODS

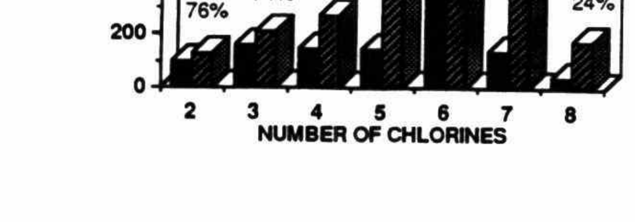


FIGURE 3: EXTRACTION EFFICIENCIES OF PCDD FROM FLY-ASH: "JAR" vs. SOXHLET METHODS

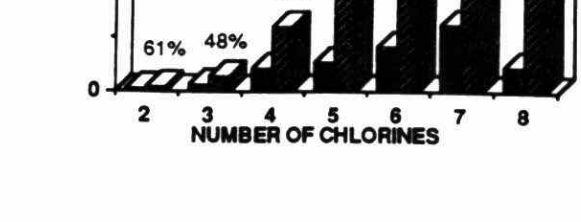


FIGURE 4: PCDF ANALYSIS OF FLY-ASH JAR EXTRACTS BY GC/MS/MS ( 3 REPLICATES)

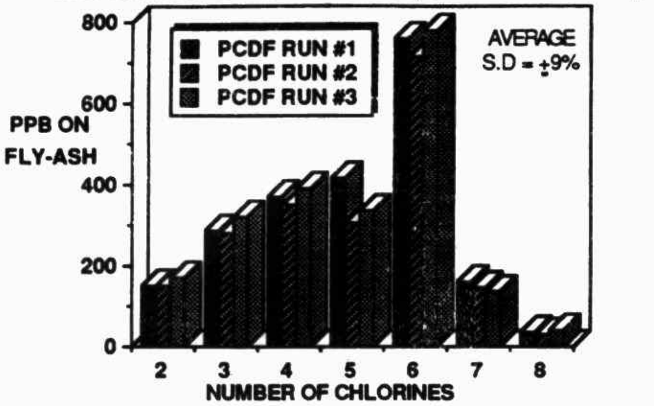


FIGURE 5: PCDD ANALYSIS OF FLY-ASH JAR EXTRACTS BY GC/MS/MS ( 3 REPLICATES)

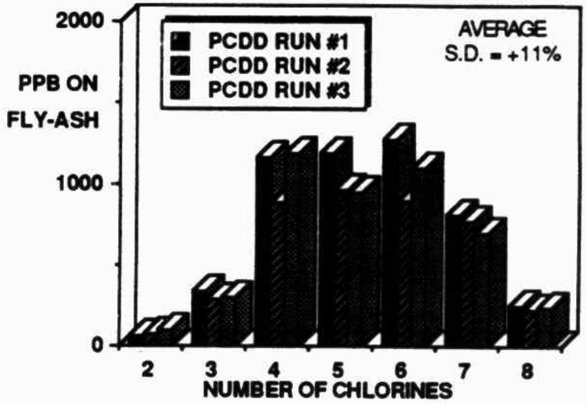


FIGURE 6: PCDD ANALYSIS OF QA/QC FLY-ASH EXTRACTS BY GC/MS/MS AND GC/MS

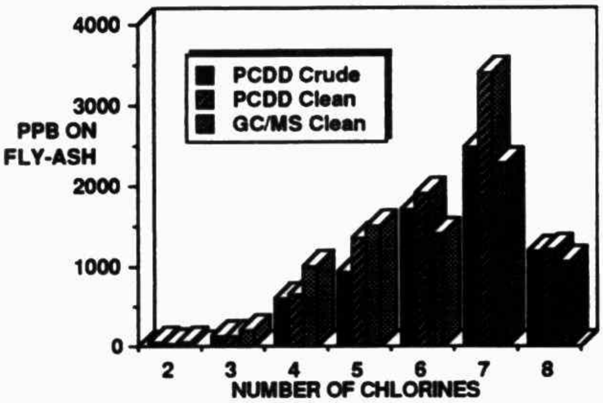


FIGURE 7: PCDF ANALYSIS OF QA/QC FLY-ASH EXTRACTS BY GC/MS/MS AND GC/MS

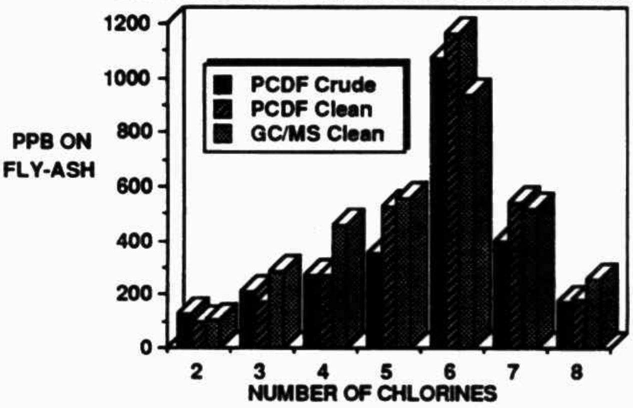


FIGURE 10: COMPARISON OF GC/MS/MS CHROMATOGRAMS FOR TCDD CONGENERS FROM JAR-EXTRACT OF FLY-ASH (TOP) AND STACK-GAS (BOTTOM)

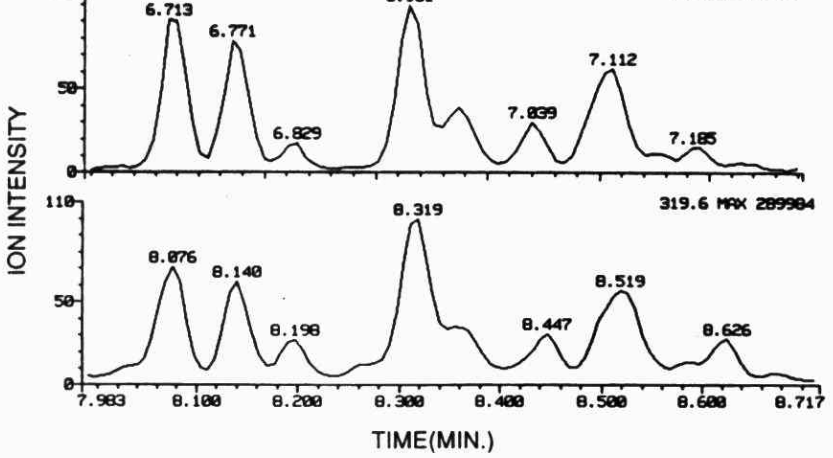


**FIGURE 8: MODIFIED MM-5 STACK-GAS SAMPLING CARTRIDGE**

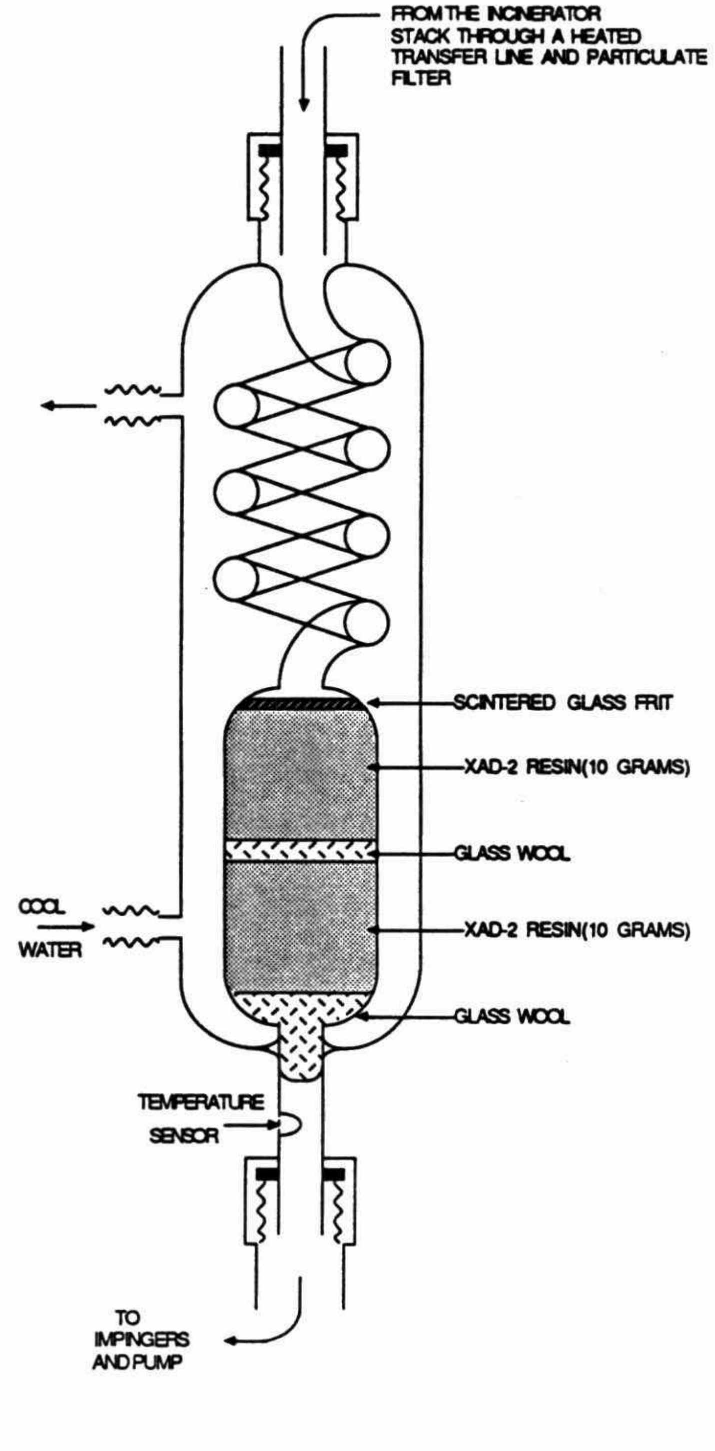


**FIGURE 9: SCHEME FOR THE JAR-EXTRACTION OF XAD-2 STACK-GAS SAMPLING TRAP**

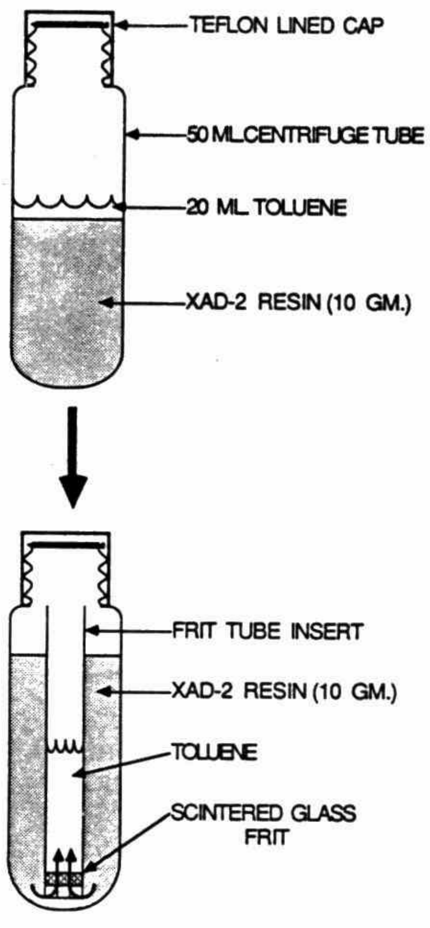


FIGURE11: PCDD & PCDF FOUND IN MUNICIPAL REFUSE INCINERATOR STACK GAS (AVG.4 RUNS)

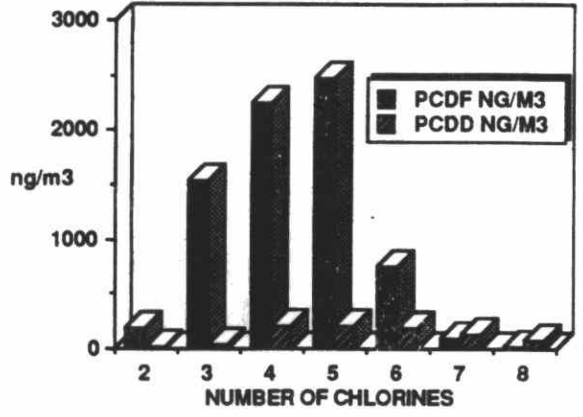


FIGURE 12: PCDD & PCDF IN SEWAGE SLUDGE INCINERATOR STACK GAS (AVERAGE 4 RUNS)

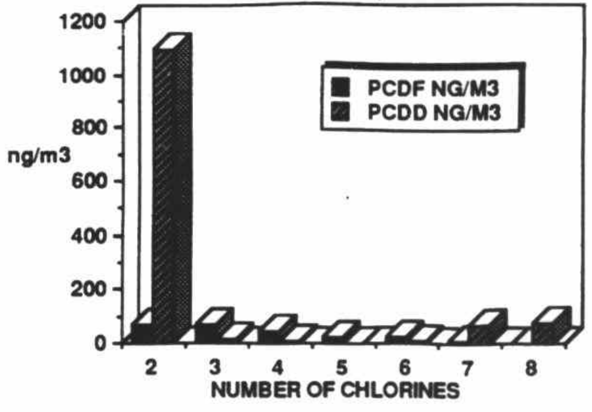


FIGURE 13: PARTITIONING OF PCDF IN MRI TRAIN; PROBE VS. "TRAP" ( AVG. 4 RUNS )

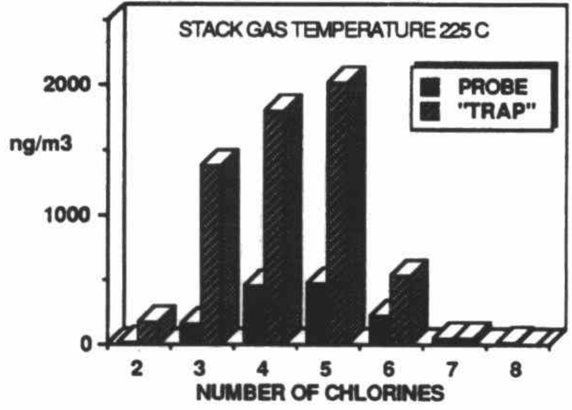


FIGURE 14: PARTITIONING OF PCDD IN MRI TRAIN; PROBE VS. "TRAP" (AVG.4 RUNS)

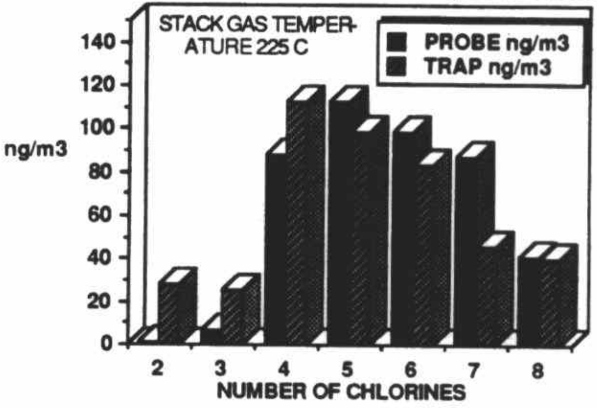


FIGURE 15: PARTITIONING OF PCDF IN MRI TRAIN; RATIO OF PROBE TO "TRAP" ( 4 RUNS)

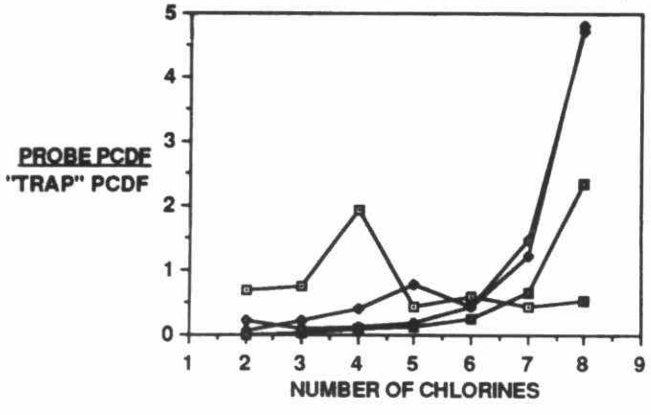
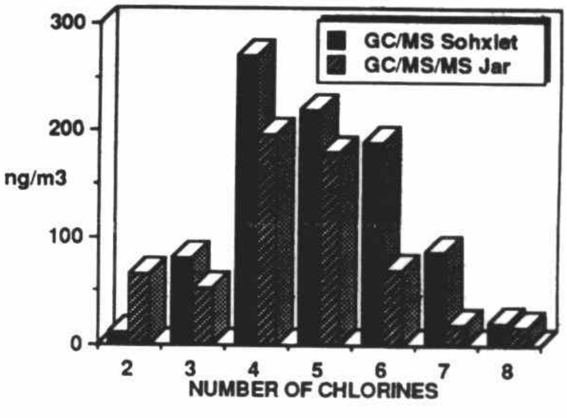


FIGURE 16: PARALLEL TRAIN PCDD LEVELS BY "JAR"-GC/MS/MS VS. SOXHLET-GC/MS



# RETROSPECTIVE CORRELATION SPECTROSCOPY AND ITS APPLICATION TO ATMOSPHERIC MONITORING

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## INTRODUCTION

Correlation spectroscopy is a powerful tool for the detection, monitoring, and measurement of concentrations of trace atmospheric species. The correlation between the intensity profile of a field spectrum and that of a "mask" which models the spectral "fingerprint" of the molecular trace species in question is a measure of the trace species concentration. The application of the method of correlation spectroscopy to environmental problems is an excellent example of technology transfer from fundamental research to important analytic application fields. The method was first proposed and used by the astronomers Bottema, Plummer, Strong and Zander (1,2) for diagnostic applications of astronomical spectra from balloon-borne payloads. Barringer and colleagues (3,4) developed the method for environmental applications.

Two types of correlation spectrometer have been developed: dispersive and non-dispersive, and early versions of both types of instrument were analogue in nature. Dispersive correlation spectroscopy as originally proposed, and as developed into the COSPEC instrument by Barringer Research Ltd, involves phase-sensitive detection of the total signal from that part of a field spectrum which passes through a focal-plane mask (of alternate transparent and opaque regions which is an analogue representation of the absorption spectrum of the species of interest) regularly oscillated in the direction of the dispersion.

In the nondispersive instrument, which is a modern deriviatve of R.W.Wood's (5) early experiments in resonance radiation, extended by Luft (6) and Goody (7), the analogue mask and the effective dispersion is achieved by using a chopper gas cell containing the vapour of the trace species to be sensed remotely. Ward and Zwick (8), then at Barringer Research Ltd., developed a field nondispersive instrument called GASPEC, the principle of which is, from their paper:

" The main feature is a pair of gas cells. The sample cell contains a judiciously chosen quantity of target gas to be sensed while the reference gas cell contains a spectrally inactive gas. The optical depth of the target gas in the sample cell is optimised for a maximum production of the modulation of the target gas energy and average transmission. Inmcoming radiation characteristic of the target is selectively filtered by being absorbed in the sample cell, but is readily transmitted through the reference cell. Therefore the radiance transmitted through the sample cell is largely independent of the presence of target gas signature in the received spectrum whereas the radiance transmited through the reference cell is strongly dependent upon the presence of the target gas. The difference between the spectral transmittance of the two cells is thus a sensitive indicator of the amount of target gas signature in the radiation received by the sensor."

GASPEC was a derivative of the Barringer bread board model of a satellite borne non-dispersive gas filter remote sensor used by Riechle (9) of NASA as part of the MAPS (Measurement of Air Pollution from Satellites) instrument flown as part of the OSTA satellite payloads on Shuttles STS-2 (1981) and STS-41G (1984) for global measurements of the atmospheric mixing ratio of carbon dioxide in the middle and upper troposphere. This is part of the new thrust in international earth and atmospheric studies in which a global view is taken of most aspects of the earth as a system.

GASPEC and the NASA instrument were analogue instruments based on the technologies of the mid-1970's, and extreme engineering care had to be taken to compensate for drifts in the analogue signal.

In this paper a brief discussion is made of our recent work to place both of the correlation spectroscopy methods on a digital basis.

## 2: RETROSPECTIVE DISPERSIVE CORRELATION SPECTROSCOPY

### 2.1: Principle of the Method

One of the limitations of the analogue method is that it calls for the use of a specific analogue mask to be installed in the spectrometer for each "target" species. Thus searches for more than one species at a time are not easily carried out. To overcome this, the basic concept of the retrospective method was proposed (11), (12). In this method, the field spectrum (of for example a smoke plume against skylight (10)) is recorded digitally and stored on diskette or tape, for later retrospective interrogation by any suitable digital mask functions, each one of which is representative of the absorption spectrum of a trace species of interest. The numerical mask can either represent the true absorption cross section spectrum of the species in question, or can represent a series of alternating transparent and opaque regions (similar to analoge correlation mask).

If I(L) is the incident radiance of the field spectrum where I is intensity and L is wavelength, and M(L-Z) is the mask function displaced in register by Z, then the correlogram between the mask function and the field spectrum is:

$$C(z) = \int I(L)\ M(L-Z)\ dL \qquad (1).$$

Under optically thin conditions:

$$I(L) = I_o(L) \exp[-N\ X\ \sigma(L)] = I_o(L)\ [1 - N\ X\ \sigma(L)] \qquad (2)$$

where X is the path length of absorber, N is its number density and $\sigma(L)$ is the absorption cross section. In short wavelength increments in the visible region of the spectrum $I_o(L)$ does not very very rapidly and may sometimes be treated as a constant. Thus (1) is

$$C(Z)/I_o = A - NX\ F(Z) \qquad (3)$$

where $A = \int M(L-Z)\ dL$ = Total mask area (4)

and $F(Z) = \int \sigma(L)\ M(L-Z)\ dL$ (5)

which is the convolution between the optical cross section and the mask function. Equation (3) provides a new direct method for determination of the column density NX of absorber. A plot of $C(Z)/I_o$ vs F(Z) has an intercept of A and a slope of NX. For many absorbers of interest $\sigma(L)$ is known from the literature, and $I_o$ is in principle known from field circumstances. It is this possible retrospectively to calculate C(Z) and F(Z) from digital measurements and thereby to determine NX. In cases where $I_o(L)$ is not constant, eg in the UV near 3000A, it is known (10) and can be is included in each of the integrals of equations (4) and (5). During the second year of the grant, equation (3) was modelled analytically using simple reasonable functional forms for $\sigma(L)$ and M(L-Z), and a function form for C(Z) determed in terms of error functions. This work has been reported previously (11) (12). During the past year the diagnostic implications of the method have been more rigorously investigated as reported below for a real molecular case and compared with laboratory measaurements.

The practical diagnostic utitlity of equation (3) has been confirmed.

### 2.2: Analytic and Digital Studies

The earlier idealised model studies (12) have been greatly extended by Mr. Amani. They have treated a) three types of case of non constancy of $I_o(L)$ (Linear, Exponential and Planckian variation with wavelength), b) more realistic models of o (L) particularly for $SO_2$ than the equally spaced gaussian comb used in the earlier work, and c) a wide variety of mask functions. These analyses are be reviewed in detail in the oral presentation of this work. When there is a strong L-dependence of $I_o$, equation (3) becomes somewhat more complex, and C(Z) is linear with respect to a sum of functions including the convolution of $\sigma$ and M, and others which arise from the functional form of $I_o(L)$. In principle this case is no more difficult to use than the more idealised equation (3). The actual cross section of $SO_2$ taken from the literature, and from our laboratory measurements was modelled analytically in this work. It was found that the greater the separation of spectral features (the greater the resolution) the better the method works. Ideally the interfeature separations should be greater than the feature widths.

### 2.3 Test of the diagnostic method against laboratory measurements.

Dr. Mandelman set up one of our digital scanning spectrometers in the laboratory. It was equipped with a Hamamatsu UV photomultiplier. A deuterium continuum lamp was used to provide $I_o$, and an $SO_2$ cell placed in the parallel pre-slit beam. A suitable vacuum system for $SO_2$ handling was set up which allows for fractional distillation of solid/liquid $SO_2$ to remove high boiling point impurities. Using this equipment Dr. Mandelman provided a digital database of I(L) vs L for a number of $SO_2$ concentration levels. Using the digitised spectra and digitised $\sigma$(L) values for $SO_2$, an extensive study of the diagnostic application of equation (3) was made by Mr.Amani. Much work went into mask optimisation. The predicted straight line plots were obtained whose slopes were consistent with the experimental values of NX to a few percent. This confirmation was very encouraging. More work is needed to develop the method further for routine field applications. This should be straightforward. Similar work needs to be done for the case of other species of interest (eg $NO_2$ whose $\sigma$ (L) does not have many well separated feastures.

## 3: GAS FILTER NON-DISPERSIVE CORRELATION STUDIES

Two non- dispersive gas filter digital correlation instruments, GASCOFIL (which is small, hand held and non-scanning) and GASCOSCAN (which is a larger spectral scanning instrument) have been developed by Mr. Morrow as passive, owd folded path active remote sensors of trace atmospheric constituents with the support of NSERC strategic grants. Depending on sensor/ filter combinations they operate from the 3000A UV cut off to 9 microns. Each is completely computer operated. Small microprocessor circuits act as controllers of the rotation of the gas call filter wheels, as controllers of the spectral scan, as controllers of the data collection, storage, real-time display and analyses. In the oral paper these instruments will be described, and measurements taken in field situations will be presented and discussed.

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# MEASUREMENT OF ATMOSPHERIC GASES BY TUNABLE DIODE LASER ABSORPTION SPECTROMETRY

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## Summary

The advantages of Tunable Diode Laser Absorption Spectrometry (TDLAS) for measuring trace atmospheric gases are universality, positive identification, good sensitivity and rapid response time. An instrument has been constructed which can measure two gases simultaneously under automatic computer control with detection limits better than 100 parts per trillion and with response times better than 5 minutes. Procedures have been established for the measurement of NO, $NO_2$, $HNO_3$, $NH_3$, $H_2O_2$ and HCHO. These species have been measured under a variety of conditions in smog chambers and in ambient air from mobile laboratories and from aircraft.

## 1. INTRODUCTION

Tunable diode laser absorption spectrometry offers an attractive method for atmospheric measurements (1). Almost all gases of atmospheric interest absorbs in the 2 to 15 micron region. The very high spectral resolution of tunable diodes permit selection of a single rotational-vibrational line which makes interferences from other gases very unlikely. If an accidental interference should happen to occur it can readily be identified by a change in line shape and another line can be chosen. Unequivocal proof of the absence of interference is obtained by measuring the concentrations at 2 different lines. The probability of identical interferences at 2 different lines is vanishingly small (2).

To get the desired sensitivity and detection limit a long absorption path can be obtained by using a multi-pass White cell. The absorption line can be scanned in a fraction of a second and the response time of the measurement is normally limited by the residence time of the sampled gas in the White cell which is typically a few seconds.

## 2. DESCRIPTION OF THE INSTRUMENT

Figure 1 shows the schematic of a TDLAS system designed to operate in field conditions from a mobile laboratory. Each of the two cryostats has a laser source assembly containing 4 laser diodes. One laser diode from each assembly can be selected to permit a choice of two gases to be measured simultaneously. The emitted radiation from each of the selected diodes is scanned over the selected line by changing the current through the laser.

The laser beam from each head is focussed by a lens and then directed to a selection mirror, S which flips back and forth to permit the beam from each of the diodes to enter the White cell in turn.

The $45^0$ angle of the entrance window to the White cell splits the laser beam. Most of the beam passes through the window into the White cell but about 5% is reflected through a cell containing high concentrations of the target gases onto a separate HgCdTe detector. The output from this detector is used to lock the laser radiation wavelength to the center of the absorption line.

The beam enters the 0.84 meter White cell, lined with Teflon, and undergoes 40 passes before exiting to the detector. Absorptions as low as $10^{-5}$ can be measured. With a total path length of 40 m this corresponds to detection limits in the range 0.1 to 0.3 parts per billion (1 ppbv = $10^{-9}$) for most atmospheric gases.

Sampled air enters through an inlet, flows down the tube and is exhausted at the other end. A restriction at the inlet end and a servo valve at the exhaust end maintains the flow rate and the pressure in the cell constant. Keeping the cell pressure at 25 Torr reduces pressure broadening of the absorption line to increase the sensitivity and to minimize the possibility of interferences from other gases.

The spectrometer is operated in the frequency modulated (2f) mode which results in an increased signal-to-noise ratio compared to using direct absorption.

The unit operates under computer control. Laser temperature and current selections for each species are input to the computer. The system is then switched to automatic mode and can operate unattended for at least 24 hours.

The computer signals the selection mirror to position itself so that one of the beams enters the White cell. A ramp voltage is then produced with 128 steps over a range just wide enough to encompass the absorption feature used for the measurement of species A. The line is scanned under computer control at a rate of approximately 10 Hz for 3 seconds (the approximate residence time of the gas in the White cell) and an accumulated "2f" line shape is acquired.

At the end of the 3 sec averaging period the signal on the reference channel, D2 is checked. If the reference channel indicates that the line is not exactly at the center of the 128 step ramp the computer adjusts the laser temperature to bring the line back to the center.

The selection mirror is then commanded to bring the second beam into the White cell and the measurement procedure is repeated for species B. Each species is thus measured once every 6 sec. Data is accumulated in this way for a period of, for example, 3 min providing an average value for the "2f" line shapes over that time frame. The data set, which provides one mixing ratio for each species, is then stored on the computer disk.

The computer manipulates the solenoid valves and flow controllers to perform measurement and calibrations sequences automatically. In a typical sequence the valve system is commanded to flow either zero or scrubbed, ambient air through the White cell. Background spectra of the components are obtained by scanning over the wavelength regions of the selected absorption lines. A typical example is shown in Figure 2a.

Calibration gas is then added to the zero (or scrubbed) air flow and allowed to stabilize for approximately 1 min. An averaged calibration spectrum is then obtained for 3 min which also serves as a reference spectrum and the raw data is archived (Figure 2b).

The background spectrum is subtracted (channel by channel) from the reference spectrum. This procedure removes any frequency dependent structure in the background and reference spectra which remains stable for the averaging period . The result of this subtraction is shown in Figure 2c.

Valves are then reset to admit ambient air, and after another stabilization period an ambient air spectrum is acquired for 3 min (Figure 2d) and the background spectrum subtracted (Figure 2e). The net ambient spectra is least square fitted to the net reference spectrum. The same calibration sequence is followed for both gases being measured.

The frequency of calibration and the time duration of the various stages of the sequences can be altered by the operator. The choice depends on the mixing ratios of the species being measured; low ambient concentrations require more frequent determination of background and reference spectra.

## 3. MEASUREMENT REQUIREMENTS

The use of the system to measure an atmospheric component has 3 requirements: (1) selection of a laser and an absorption feature; (2) establishment of a calibration procedure; (3) ensurance of sampling integrity.

The selection of a laser and an absorption feature represents a compromise between the absorption line strength, the characteristics of the laser emission at the wavelength of the line, and absence of interferences at this wavelength from other atmospheric gases.

The calibration philosophy calls for the addition of a known amount of the calibration gas to the sampled air stream at the entrance to the sampling line. The concentration of the "spike" is chosen to provide an increase in measured mixing ratio comparable to the mixing ratio of the gas in the ambient air. In this way, any surface effects that may occur will be the same for the sampled and spiked air and should therefore be compensated.

Finally, tests are performed to ensure sampling integrity. The air sample is continuously drawn through a short length of 6 mm teflon tubing and the pressure reduced by an all Teflon needle valve. The flow rate of the sampled air is typically 6 SLM giving a residence time of a tenth of a sec in the sampling line and 3 sec in the White cell. The response time of the system is defined as the time required for the signal from an addition of the species to drop to 90% of its value when the source is removed or the time for the signal to reach 90% of its final value when the source is introduced. Response times greater than the residence times are indicative of interaction of the gases with the surfaces of the sampling system and has been observed for $HNO_3$, $NH_3$ and $H_2O_2$. In those cases careful studies were performed to assess the nature of the surface processes and sufficient sampling time is allowed for the system to reach steady state with the ambient air.

These procedures have been established for NO, $NO_2$, $HNO_3$, $NH_3$, $SO_2$, HCHO, and $H_2O_2$ and measurements of these gases have been made under field conditions and in smog chamber experiments.

## 4. EXAMPLES OF ATMOSPHERIC MEASUREMENTS

Bottled gas of NO in $N_2$ at a concentration of a few ppmv, traceable to a National Bureau of Standards determination, has been used as the calibration source for NO. The response time of the system for this gas is the residence time in the cell of 3 sec. The minimum detection limit, MDC, is defined as a signal to noise of one, is better than 100 pptv (1 pptv = $10^{-12}$).

A commercial permeation tube is used for the calibration source for $NO_2$. The permeation rate is determined by weight loss and also checked by $NO/O_3$ titration with NO then serving as the calibration standard. The response time and MDC are 3 sec and 100 pptv.

Figure 3 shows typical diurnal variations of $NO_2$ concentrations measured at a relatively clean rural site. Peak concentrations of about 3 ppbv decrease during the day as photochemistry proceeds and increases again after sunset, (~ 8 p.m). Oxidation by $O_3$ decreases the $NO_2$ concentration during the night.

Figure 4 shows an example of the higher concentrations observed in a relatively polluted urban environment. Diurnal variations are similar with mixing ratios as high as 150 ppbv observed. This figure also shows an interesting example of how the TDLAS technique can be used to serve as a reference method against which other, less definitive methods can be compared. Simultaneous measurements were also made with a LUMINOx instrument based on chemiluminescence from a luminol solution. The two methods agree except for two nights when the pollution levels were quite high. On these occasions the luminol method gave values some 35% higher than the TDLAS method, indicating that the luminol method was also responding to some other species present under these conditions at night.

The line strengths for $HNO_3$ are about 4 times lower than for $NO_2$ resulting in an MDC of about 300 pptv. The calibration source for $HNO_3$ is a specially designed permeation device in which the carrier gas is passed through a Teflon tube immersed in a solution of $HNO_3$ and $H_2SO_4$ acids. The permeation rate is determined by potentiometric titration with standard NaOH solution. Sampling integrity is the major problem with measuring $HNO_3$ since this gas is highly polar and absorbs readily on most surfaces. The response time of the system decreases with increasing flow rate of the sampled air through the system and is about 5 min for flow rates greater than 6 standard liters per minute (SLM).

Figure 5 shows typical diurnal behavior of measurements of $HNO_3$ taken at a rural site. The concentration peaks in the early afternoon and decreases rapidly in the late afternoon and evening, indicative of the rapid dry deposition rate of this species. The rapid dry deposition is also demonstrated in Figure 6 which shows difference between measurements made at heights of 1 m and 8 m above a snow surface.

The line strengths for $NH_3$ are high permitting detection limits of 100 pptv. But, like $HNO_3$, $NH_3$ is a "sticky" molecule requiring air flows through the system of at least 6 SLM to provide response times of 5 min. The calibration source is a gas cylinder containing $NH_3$ in $N_2$ having a concentration of a few ppmv which is checked periodically by potentiometric titration with standard acid.

Figure 7 shows simultaneous measurements made of $NH_3$ and $HNO_3$ at a rural site. The measurements show indication of anticorrelation which suggests mutual neutralization. However the product of the concentrations is much less than the equilibrium values with solid $NH_4NO_3$ and it is more likely that the anticorrelation results from mixing of different air masses.

For calibration of $H_2O_2$ carrier gas is flowed through a 4 m coil of polyethylene immersed in a 50% solution of stabilized $H_2O_2$. The permeation rate of this device was determined by a modification of the colorimetric $TiCl_4$ method. The response time for $H_2O_2$ is about 40 sec; apparently some conditioning time with the apparatus is required for this species but much less than is required for $HNO_3$ or $NH_3$.

The relatively weak line strengths for hydrogen peroxide limits the detection of hydrogen peroxide to between 300 and 600 pptv for 5 min integration times with the system described above. Measurements made with this system showed that the $H_2O_2$ mixing ratios in ambient air is generally less than this detection limit. Modifications were therefore made in the system which consisted of increasing the base path of the White cell to 1.5 m and using improved, astigmatic entrance and exit optics. These modifications permitted a total path length of 200 m to be achieved with a concommitant improvement in detection limit. With the new system detection limits for $H_2O_2$ of at least 100 pptv were obtained. The improved system gives detection limits for $NO_2$ and $HNO_3$ of 25 and 75 pptv respectively.

Figure 8 shows an example of $H_2O_2$ measurements made under relatively polluted conditions and Figure 9 shows measurements made under relatively clean conditions. Both studies showed similar diurnal variations with concentrations reaching maxima in the afternoon and minima during the night. The mixing ratios reach higher values in the clean air conditions.

The calibration source for formaldehyde is a Teflon permeation device containing paraformaldehyde. The permeation rate is determined by a modification of the chromotropic acid colorimetric method. No sampling problems are encountered with this gas and the response time of the system is the 3 sec residence time. The detection limit for HCHO with the improved system is better than 50 pptv.

Figures 10 and 11 show examples of $H_2CO$ measurements under the same conditions as shown for $H_2O_2$ in the preceding figures. For the polluted site the peak concentrations were observed near local noon. For the relatively clean site the peak occurred somewhat later in the afternoon and was considerably lower than for the polluted site.

The TDLAS system has also been used to measure these gases in smog chambers. A modified version of the system in which a liquid $N_2$ Dewar was substituted for the helium cryostat was flown successfully in and above the boundary layer over the eastern Pacific to measure $NO_2$ and $HNO_3$.

## 5. CONCLUSIONS

The tunable diode laser absorption spectrometer has been shown to be a very versatile system for measuring trace atmospheric gases from mobile laboratories and from aircraft under a number of ambient and smog chamber air conditions. Its high specificity, good sensitivity and rapid response time makes it a very suitable standard against which other less definitive methods can be compared.

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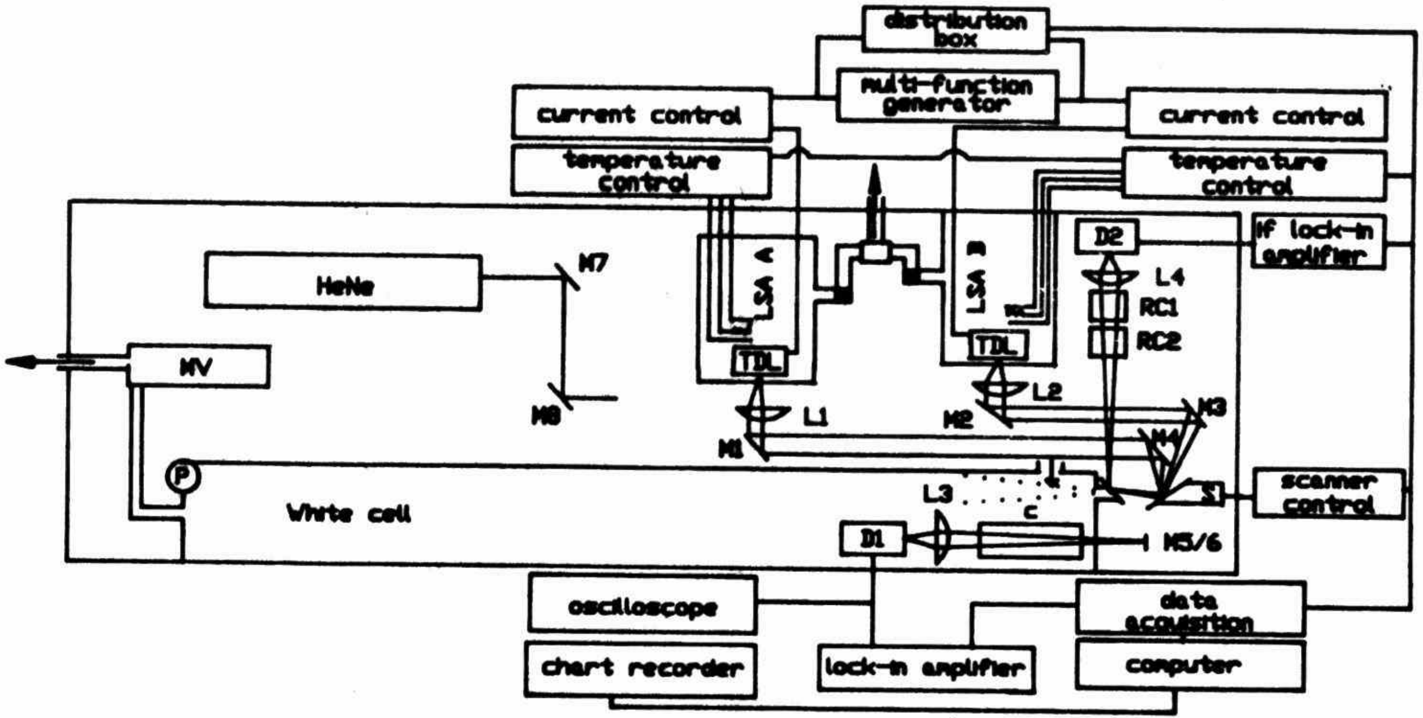
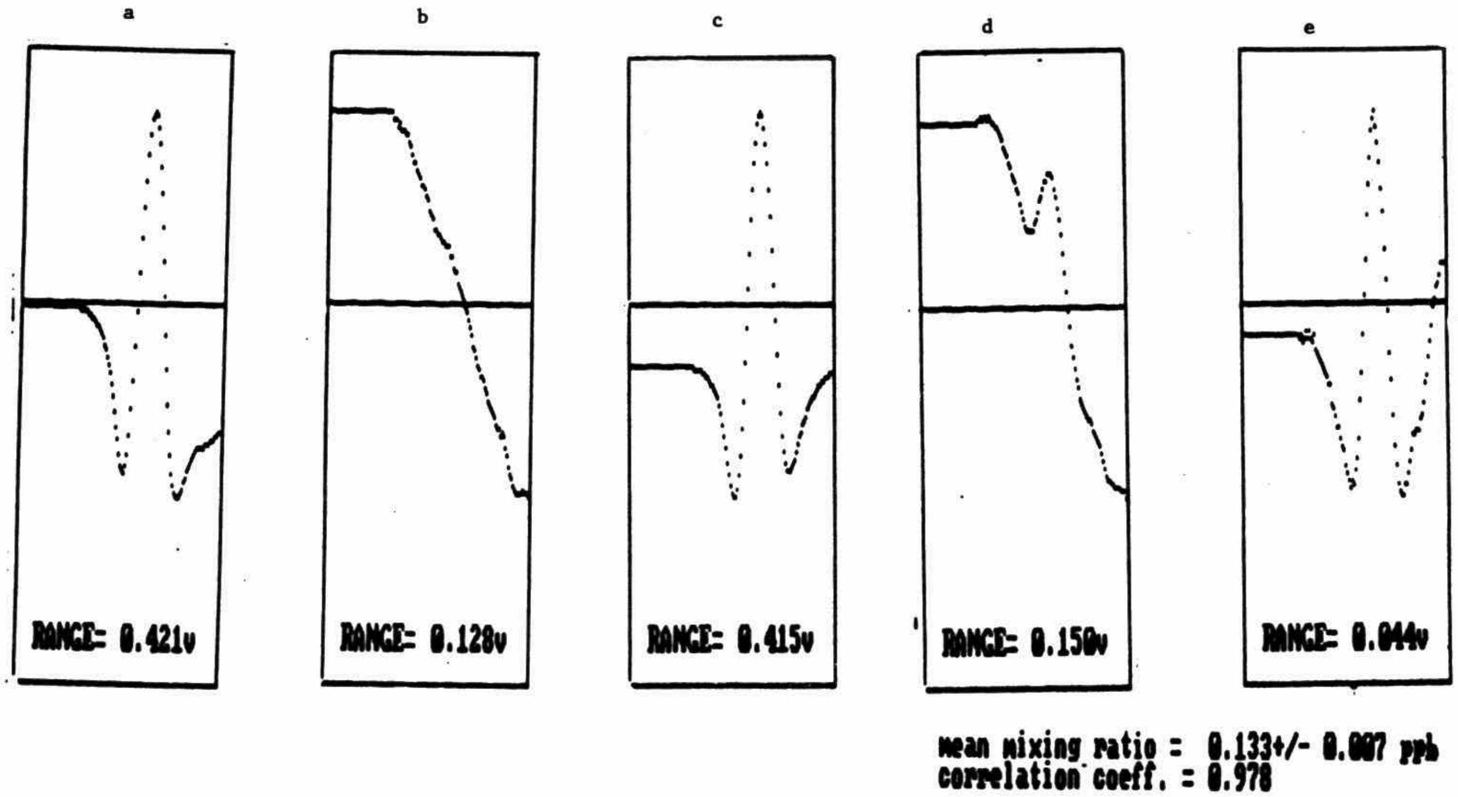


Fig 1: Schematic of the optical system and control electronics



mean mixing ratio = 0.133+/- 0.007 ppb
correlation coeff. = 0.978

Fig 2: Computer screen printouts for $NO_2$ aircraft measurements.
(a) raw calibration spectrum; (b) raw background spectrum; (c) calibration spectrum, background subtracted; (d) raw ambient spectrum; (e) ambient spectrum, background subtfacted.

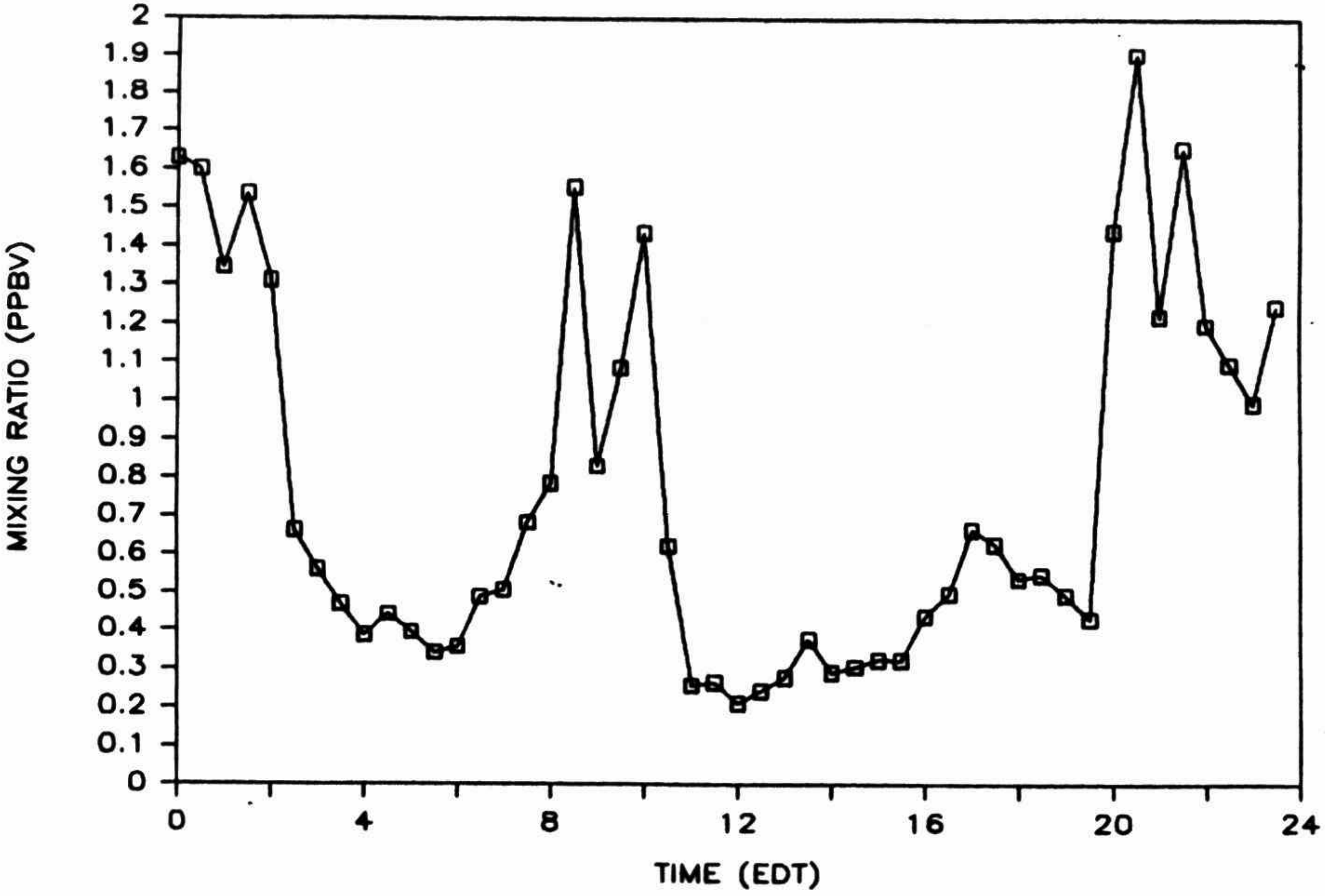


Fig 3: 30 min average $NO_2$ measurements made at Cold Creek, Ontario on June 25, 1985

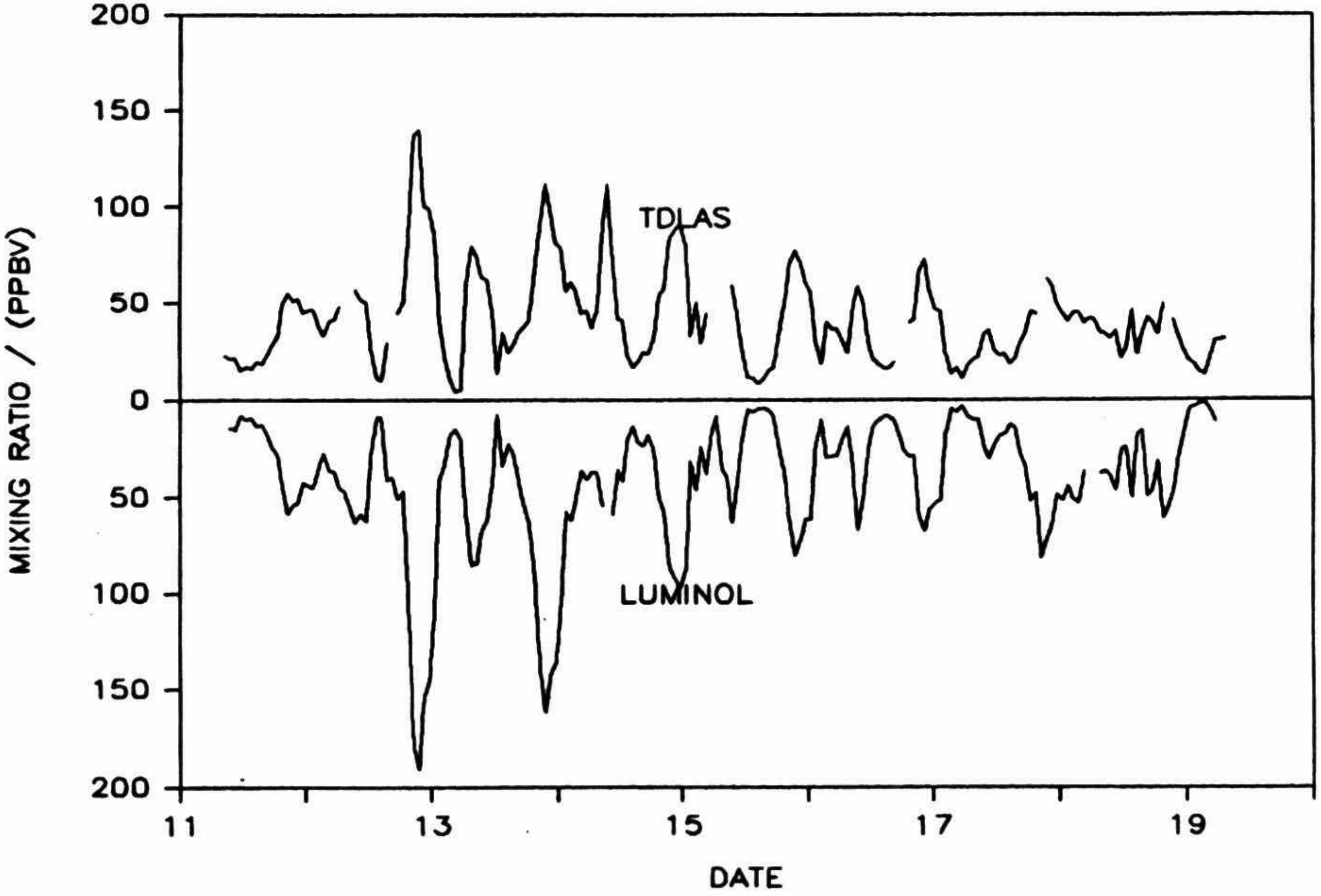


Fig 4: Comparison between 1 hr $NO_2$ measurements made simultaneously by the TDLAS and the LUMINOX instruments at Claremont CA during September 1985.

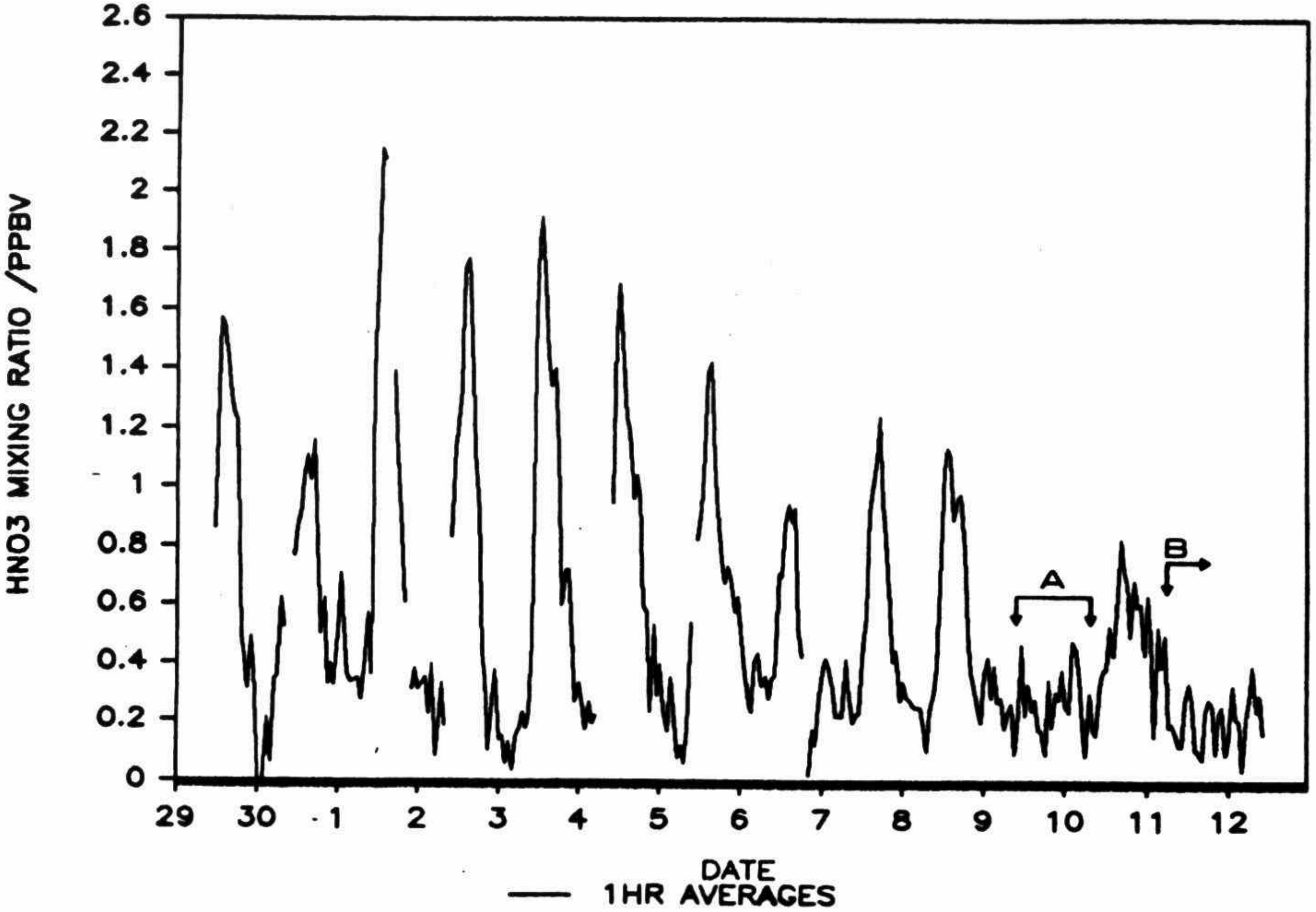


Fig 5: hourly average $HNO_3$ measurements at Rayleigh NC Sept 29 - Oct 12. annular denuder in front of inlet during period A, heavy rain during period B.

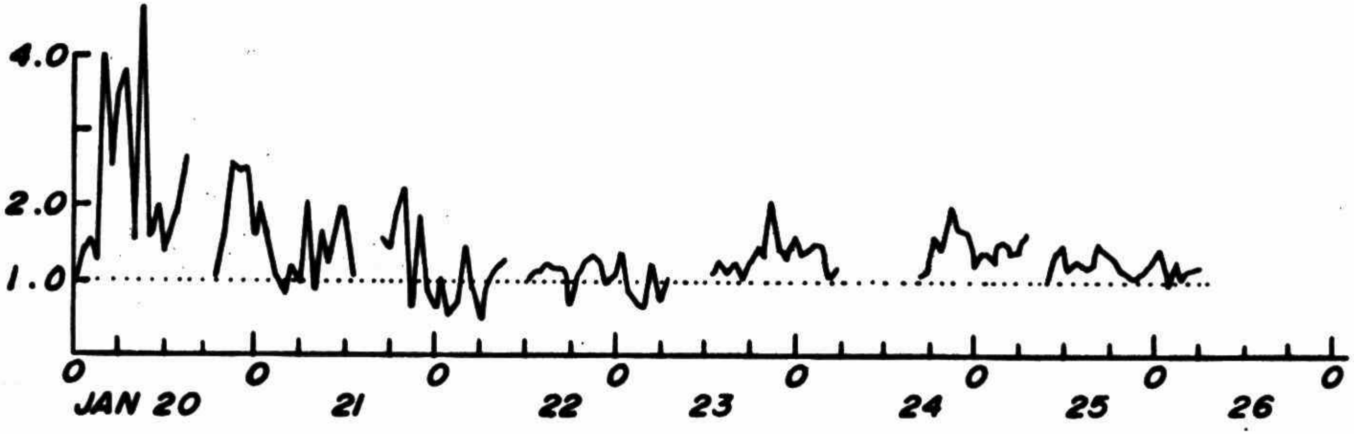


Fig 6: Average ratios of $HNO_3$ mixing ratios measured at 8 M and 1 M above a snow surface at State College, PA from Jan 20-26, 1984.

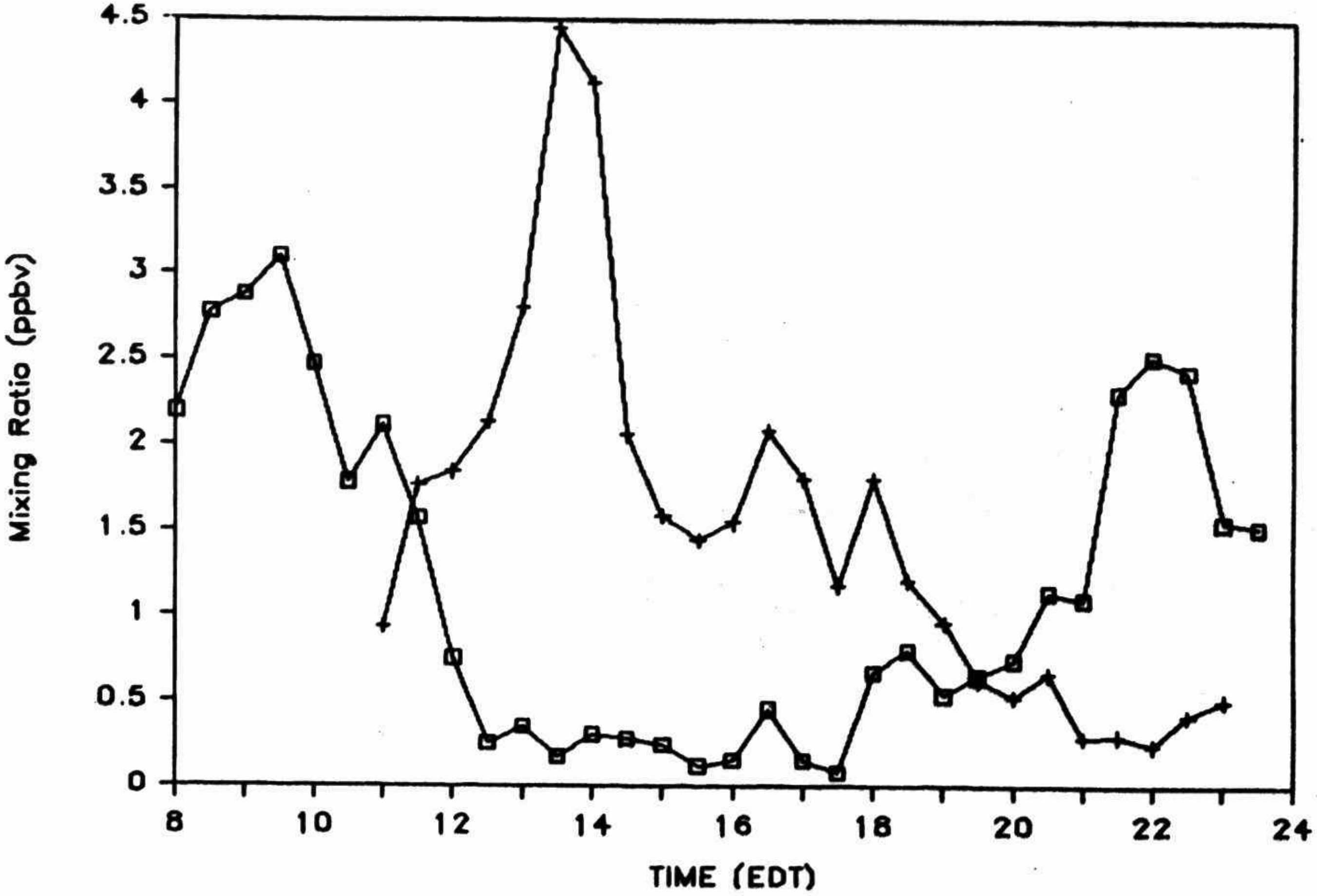


Figure 7: 30 min average mixing ratios measured at Cold Creek, Ont. July 5, 1985
+ $NH_3$ $HNO_3$

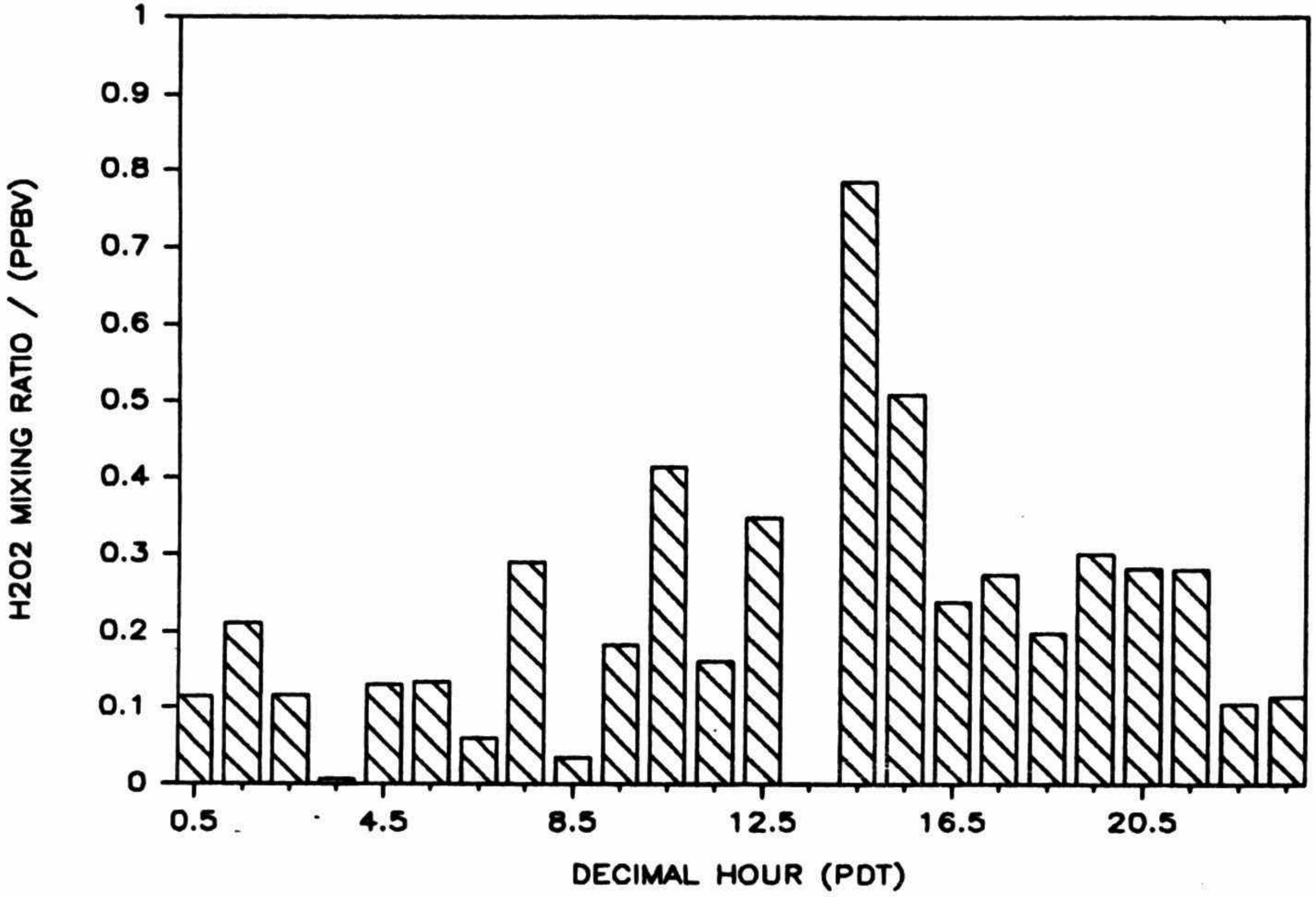


Fig 8: hourly averaged $H_2O_2$ mixing ratios at Glendora CA on Aug 12, 1986.

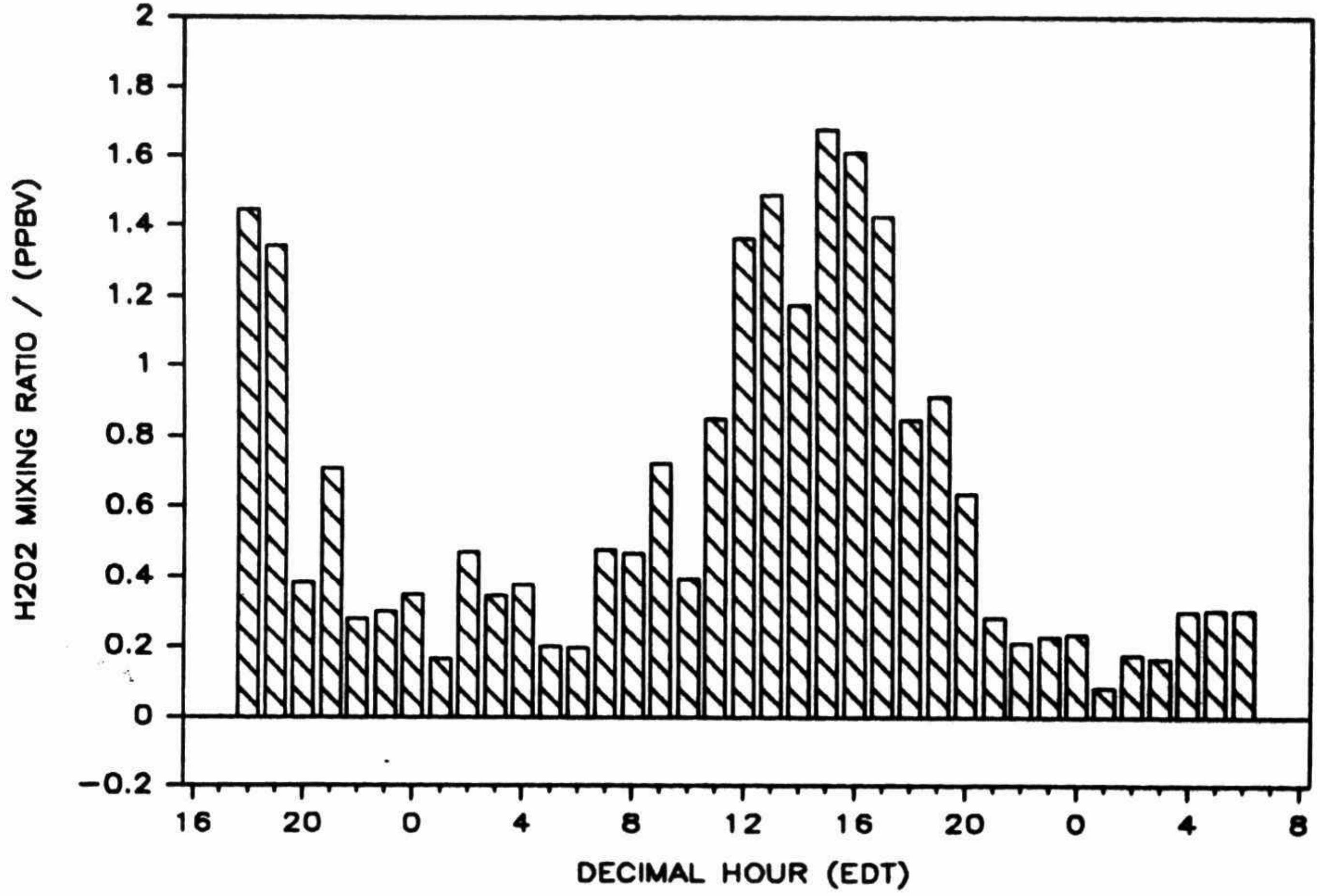


Fig 9: hourly averaged $H_2O_2$ mixing ratios of $H_2O_2$ at Rayleigh NC between June 24 and June 26, 1986

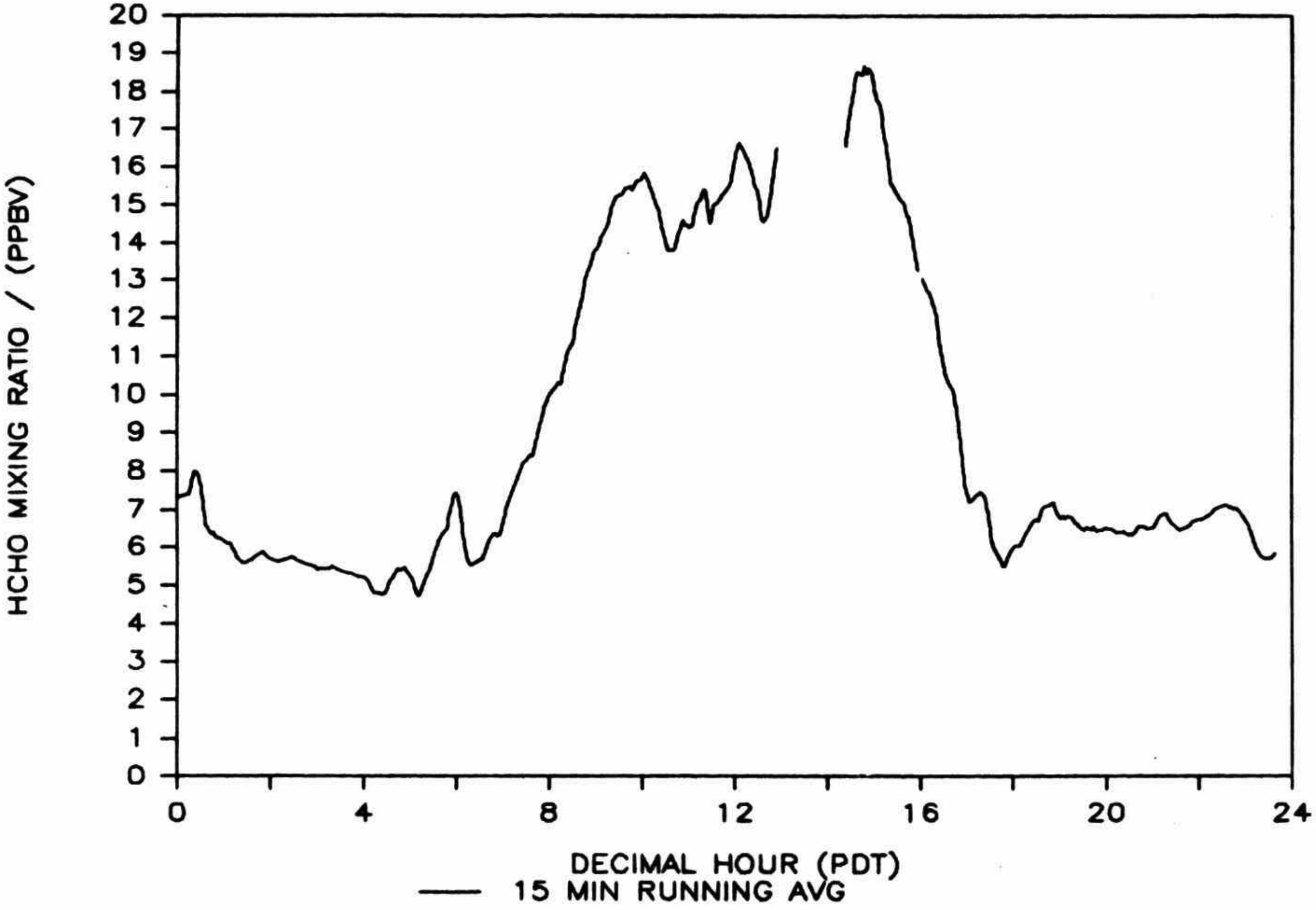


Fig 10: HCHO mixing ratios at Glendora CA on Aug. 12, 1986.

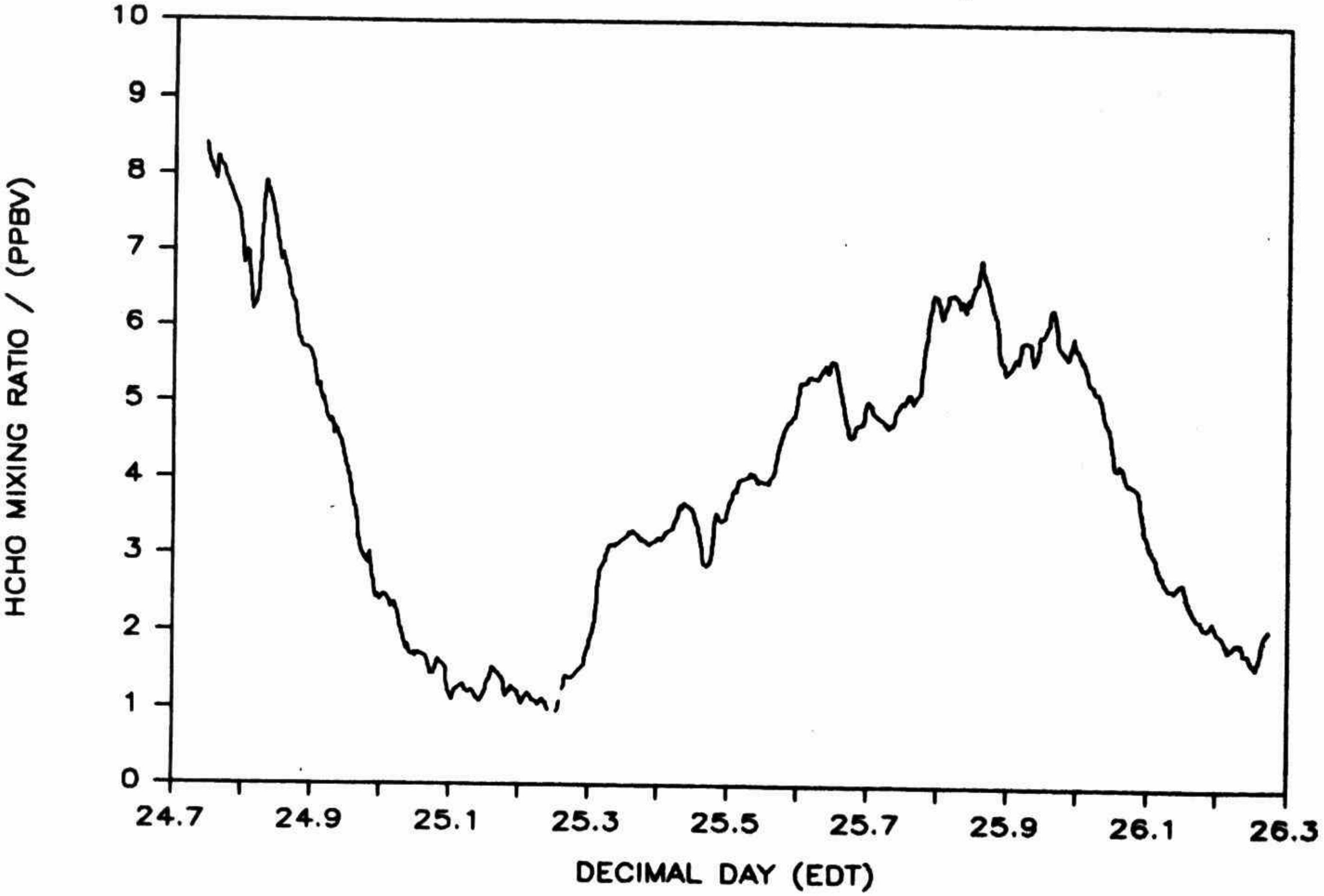


Fig 11: hourly average HCHO mixing ratios at Rayleigh NC.
Times shown are decimal days between June 24 and June 26.

# Uptake, Distribution and Clearance of Soluble Aerosols from the Human Respiratory System

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In humid atmospheres, soluble aerosols may grow in size as water vapours condense upon the particle surfaces. The growth of soluble aerosols in the humid environment of the respiratory tract will affect the degree and site of deposition in the lungs. Once deposited, the soluble aerosols are subject to different clearance mechanisms than insoluble particles, and the residence times of such particles in the lung may be very short. Since it has been shown that most of the ambient aerosol is hygroscopic (Hicks and Megaw, 1985), this has a direct bearing on the estimation of health effects due to airborne pollutants. This paper describes a study of the growth, deposition, uptake and clearance for two different soluble aerosols, mainstream cigarette smoke and sodium chloride.

## Effect on growth on total deposition

The sodium chloride aerosol was generated from a saline solution in a nebulizer (Acorn, Medic-aid Ltd., London). The aerosol was allowed to dry in a 40 litre glass chamber prior to inhalation. The size distributuion of the aerosols in the chamber was determined using a quartz crystal microbalance cascade impactor (QCM, Berkely Instruments, Model C1000-A) immediately before the subject inhaled 1 litre of the aerosol-laden air from functional residual volume. The exhaled air was diluted with dry air and sampled. Inhaled and exhaled aerosol measurements were used to determine the total lung deposition as a function of size for the NaCl aerosol. This procedure was repeated using mainstream smoke drawn from a lit cigarette using a syringe, and injected into a 40 litre glass chamber. The smoke was aged for approximately 1 minute before inhalation.

The results of the experiment conducted in 5 subjects were similar for both aerosols. The point of minimum deposition occurred at 0.1 um in contrast to 0.5 um for insoluble particles (Fig. 1). This minimum agrees with the results of others using NaCl (Blanchard and Willeke, 1983, Tu and Knutson, 1984), and with a model of NaCl deposition by Xu and Yu (1985). The results of Dautrebande and Walkenhorst (1961) record a smaller particle size of minimum deposition at 0.07 um. However this appears to be an optical diameter measurement and would equate to 0.09 um if the density of NaCl is considered.

Although the shape of the deposition curve matches past results, we found a large degree of inter-subject variability, and generally higher values of deposition than previously reported. This may be due to the subjects used or to the fact that our results are the only measurements of this type based on a mass measurement device instead of an optical particle counting method.

Since the deposition data indicated that small particles grow to larger sizes in the lung, a second experiment was conducted to determine the degree of aerosol growth in the respiratory tract. A direct measurement of the size distribution of the humidified, exhaled aerosol was taken at the mouth and compared with the aerosol after it had been dried, again using the QCM cascade impactor. For the humidified profile, the size distribution was measured one stage at a time and composite profile, was constructed from individual measurements. The time from exhalation to impaction was estimated to be less than 0.1 seconds, therefore little drying or cooling occured. The dry aerosol profile was measured once a sample of exhaled air had been dehumidified with dry air. This measurement was taken with the cascade impactor set in the normal mode and was not a stage by stage composite sample. The exhaled NaCl aerosol was found to be 4.5 times larger in mass mediam aerodynamic diameter when humidified than when dired. This growth is similar to that estimated by Ferron (1977). The humidified mainstream smoke measured 1.7 times larger than the dry size, yet with a similar minimum in deposition to NaCl.

## Effect of growth on regional deposition

The regional deposition of both aerosols was investigated using a radioactive label, 1-iodohexadecane (I-123, $T_{½}$= 13.5 hr.) previously developed from mainstream smoke (Pritchard and Black, 1984). This was adapted to label NaCl by adding it to an ethanol-NaCl solution in the nebulizer. Upon nebulizing, the alcohol evaporates and the label is retained with the dry NaCl particles. The label constitutes less than 2% of the mass of the particle and did not appear to impede the condensation of water on the particle. Although both aerosols are polydisperse the size distributions are similar.

Table 1: Summary of Regional Deposition Experiments

| Ref | Material | Tidal Volume l | Flow $l\ s^{-1}$ | Pause s | Breathing rate $min^{-1}$ | MMAD um | $\sigma g$ |
|---|---|---|---|---|---|---|---|
| 1 | 40 ml puff of smoke | 0.5 | 0.5 | 2 | - | 0.6 | 1.8 |
| 2 | 40 ml puff of smoke | 1.5 | 0.5 | 2 | - | 0.6 | 1.8 |
| 3 | 260 ml smoke in 40 l | 1.5 | 0.5 | 2 | 7 | 0.6 | 1.8 |
| 4 | 0.8 mg NaCl in 40 l | 1.5 | 0.5 | 2 | 7 | 0.7 | 1.9 |

In the first two tests the volunteer drew a 40 ml bolus of labelled smoke from the cigarette. The bolus was then inhaled in a particular breathing regime (see Table 1). In the third and fourth tests the aerosol was inhaled from the 40 litre chamber over a period of 7 breaths. Five volunteers smokers were used in the experiment. After inhalation, lung retention was followed for 30 hours using the whole-body monitoring techniques previously developed (Pritchard and Black, 1984). Retention of the label in the lung can be described by a two-phase exponential, which may be interpreted as clearance from the tracheobronchial (TB) and pulmonary (P) regions. The P and TB components of the lung deposit were estimated by a weighted least squares technique which extrapolated the two phases of the retention curve back to the time of administration (Fig. 2).

The fraction of the lung deposit in the TB region is given in Table 2. The values for the deposition of cigarette smoke are in good agreement with those previously reported (Pritchard and Black, 1984). As can be seen, there is a large and consistent inter-subject variability. The use of a non-parametric ranking test (Friedman, 1937) shows that the subjects, as well as the experiments, cannot be considered as coming from the same sample population ($p < .025$ and $p < .01$ respectively) Experiments were compared using a paired t-test.

Two effects of breathing pattern on deposition were demonstrable. There was a decrease in the relative proportion of TB deposit on increasing the tidal volume ($p < .05$). This is due to the longer residence time of the aerosol deep in the lung resulting in increased deposition by sedimentation in the P region. Also, there was an increase in TB deposition during steady-state smoke inhalation when compared to a puff inhaled in the same manner. Presumably, this is because the bolus of smoke from a puff is swept into the P region by clean air wheras smoke is constantly present in the conducting airways during steady-state breathing, resulting in greater deposition by sedimentation in this region.

Table 2: Percentage of Tracheobronchial To Total Lung Deposits (%)

| | Experiment | | | |
|---|---|---|---|---|
| Subject | 1 | 2 | 3 | 4 |
| A | 81.9 | 80.7 | 82.2 | 84.6 |
| B | 69.3 | 63.3 | 69.1 | 78.5 |
| C | 61.7 | 57.7 | 72.4 | 72.8 |
| D | 55.4 | 53.9 | 56.2 | 54.2 |
| E | 46.3 | 45.4 | 65.1 | 53.7 |
| Mean | 62.9 | 60.2 | 69.0 | 68.8 |
| +/- SE | 6.1 | 5.9 | 4.3 | 6.3 |

## Significant Conclusions of the Regional Deposition Experiment

Of primary importance is the signficant amounts of tracheobronchial deposition in every experiment. The amount of TB deposition found with insoluble particles in other experiments was much smaller. The soluble aerosols used in this experiment have deposited in this region to such a degree that the tracheobronchial component usually exceeds the pulmonary.

Table 3
Summary of Parameters by Volunteer
(Average of 7 Regional Deposition Tests)

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Average % P | 18 | 32 | 31 | 49 | 52 |
| Average % TB | 82 | 68 | 69 | 51 | 48 |
| Average P $t_{1/2}$ (hrs) | 15.8 | 16.9 | 12.6 | 12.8 | 11.6 |
| Average TB $t_{1/2}$ (hrs) | 1.9 | 1.7 | 1.8 | 1.4 | 1.3 |
| Average M $t_{1/2}$ (hrs) | 12.9 | 12.5 | 10.7 | 11.3 | 11.5 |
| Average Total Activity Absorbed (counts) | 2097 | 2478 | 3552 | 2920 | 2650 |

The TB deposit was cleared very rapidly in all cases (average TB $t_{1/2}$= 1.6 hours) and most of the deposit was cleared within five to six hours. The deposit was cleared to the gut and some components entered the bloodstream from this point. Therefore, many soluble aerosols (e.g. air particulate) may deposit heavily in the tracheobronchial region, and be cleared to the gut and/or bloodstream within a few hours. The physical insult to the airway passages may only be present for a few hours, but if toxic components exist and are not digested by gastric juices they may still make their way into the bloodstream once cleared from the lung.

On the basis of these experiments we cannot assume most deposited submicron particles will reside in the pulmonary region. However, the degree of deposition varies from volunteer to volunteer and the tracheobronchial component may consist of 35% to 85% of the lung deposit. The determining factor appears to be highly dependent on the individuals' anatomy and breathing behaviour.

The degree of deposition appears to be dependent primarily on the length of time of breath. The longer the residence time of the aerosol in the lung the greater the deposit. Therefore the aerosol deposits via a time dependent mechanism, presumably sedimentation and diffusion.

It was noted that pulmonary deposition increased when the tidal volume changed from 0.5 to 1.5 litres. Also, the tracheobronchial component was greater in the multiple breath experiments than in the single breath "bolus" experiments. Presumably this was due to the deposition, via sedimentation, of smoke during respiratory "pauses".

The pulmonary clearance rate for this label was quite rapid. Instead of clearing over a period of months or years as insoluble substances do, this label had a half-life clearance rate of 10-20 hours. The rate depends on the individual and in this set of volunteers there appreared to be a dependence on age and/or smoking history. Periodic measurements on a larger number of volunteers over a period of years could explain why the pulmonary clearance rate varies from volunteeer to volunteer.

Although the regional despostion of mainstream smoke was not identical to that of the sodium chloride aerosol (Test #3 vs. Test #4 - Table 2), the deposition measurements of the two are comparable. The range in percent pulmonary deposition measurements for mainstream deposition (Test #3) was 18-44% (Ave. 31.8%) for the sodium chloride aerosol. Therefore the deposition measurements of the two aerosols are not dissimilar. Indeed, for a study that must use human volunteers the results are remarkably close.

It is also interesting to note that the total deposition measurements revealed a similar deposition curves for both the mainstream and sodium chloride aerosols. The two curves had similar points of minimim deposition at a particle size of 0.1 um (diameter). However, the degree of deposition did vary between the two aerosols.

## Applicability of These Results of the Assessment of Environmental Particulate Deposition

The solubility of environmental particulate has been shown to be similar for many regions in the Province of Ontario. The total mass of the fine particulate has been found to be on average 60% soluble. Individual elements may differ widely in solubility but tend to show similar values from region to region in the province. Although the solublity of an element may be consistent from region to region, the total quantity of that element found airborne varies depending on the airshed being assessed.

An assessment of the size distribution of the fine particulate found it to be similar to that of the mainstream ciga rette smoke used in these deposition and growth stuides. The $D_{50}$ of the fine particulate was approximately 0.7 um with a sigma-g of 1.8 whereas the mainstream smoke had a $D_{50}$ of 0.6 and a sigma-g 1.7. Similarly the mainstream smoke was found to grow in the presence of humidity by a factor of 1.7. The data of Hicks (1984) translates to a growth factor of environmental particulate of 1.8

Therefore, environmental particulate should undergo mechanisms of deposition and clearance in the lungs, similar to those of the mainstream cigarette smoke. It is likely that large portions of the inhaled particulate will deposit in the tracheobronchial tract as well as the pulmonary region. The deposited particulate should clear from the tracheobronhical tract to the gut within hours. Soluble elements will also pass from the gut into body fluids. Similarly many of the soluble particulate components should clear from the pulmonary region by absorption into the body. The pulmonary absorption route should drastically reduce the particulate burden in the lung within a few days after deposition. Other insoluble species most probably remain in the pulmonary region to be cleared by other means (e.g. macrophage long-term clearance).

Using the data obtained on fine particulate solubility we can identify some of the elements which will clear rapidly by absorption, and others which may build-up in the pulmonary region over long periods of time (See Table 4).

If we assume these elements do not react with the lung surface and that soluble elements will move through the epithelial layer as the tracer did regional deposition experiment, then we can determine the elements which are retained in, or cleared from the pulmonary region.

Table 4: Summary List of Elemental Solubility in Ontario Fine Particulate

| Element | Percent Solubility |
|---|---|
| Aluminum | 55 |
| Silicon | 21 |
| Phosphorus | 78 |
| Sulphur | 93 |
| Potassium | 88 |
| Calcium | 67 |
| Titanium | 8 |
| Vanadium | 56 |
| Chromium | 25 |
| Manganese | 65 |
| Iron | 14 |
| Zinc | 59 |
| Bromine | 74 |
| Lead | 70 |

Most of the inhaled silicon, titanium, chromium, and iron that deposits in the pulmonary region will be relatively insoluble. Hence it is unlikely they will absorb readily into the body fluids, but instead remain in the pulmonary aveoli, to be cleared by macrophages. These elements will be subject to long term clearance mechanisms.

However, other elements are primarily soluble and should absorb into the body fluids if deposited in the pulmonary region. These elements should almost completely absorb within days of being inhaled.

Certainly, most elements cannot be classified as completely insoluble. More likely each element has an insoluble and soluble fraction. It is also important to note that different geographical areas will have different levels of particulate elements in the air. For example the air in Area A of an Ontario city has an average silicon content of 0.9 ug/m$^3$ whereas Area B has an average of 0.15 ug/m$^3$ Si in the air. Therefore particulate levels must also be taken into account when assessments are made.

Some elements may be toxic to certain body tissuses. For instance, the element lead affects the nervous system and may be absorbed by the bones. Therefore the rate at which lead is absorbed from the lung and carried to these tissues may be important to its toxic assessment.

Particulates that deposit in the tracheobronchial region should be cleared in a matter of hours. However, these elements may be absorbed through the gut and may end up in the body fluids despite their rapid removal from the lungs.

In summary, particulate solubility is important to the assessment of xenophobic substances inhaled into the body. The solubility of a species may determine its rate of transport from sytem to system. Or it may be useful in calculating the long term build-up of insolubles in the lower lung.

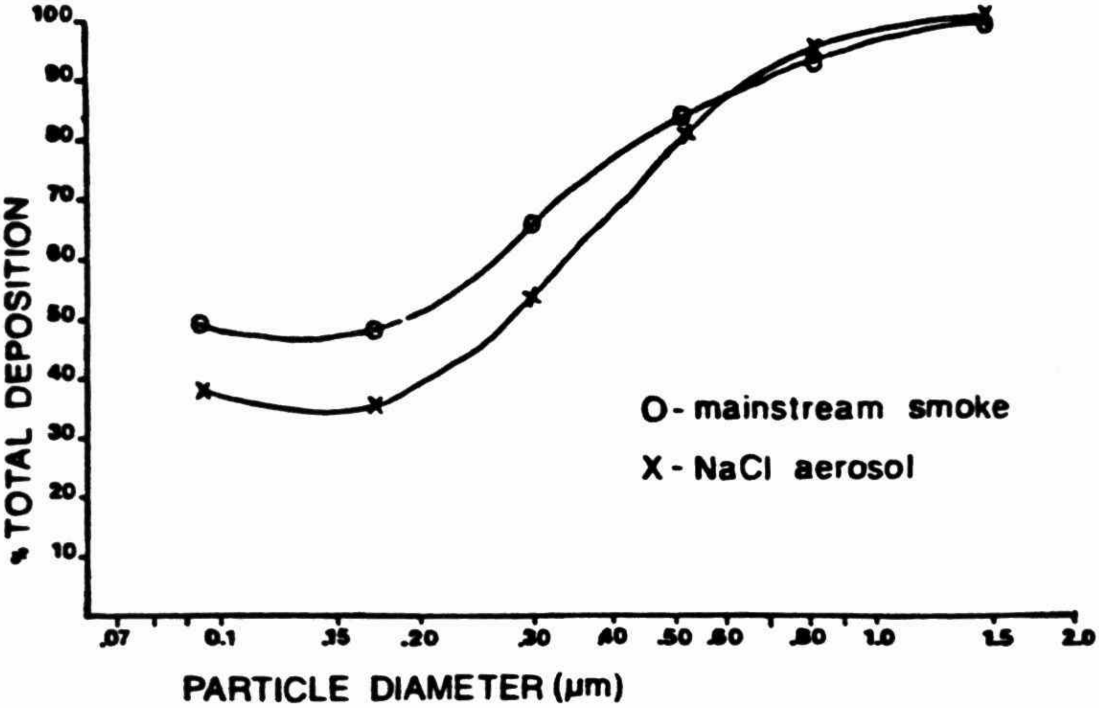

Fig. 1: Total deposition as a function of particle size for two soluble aerosols

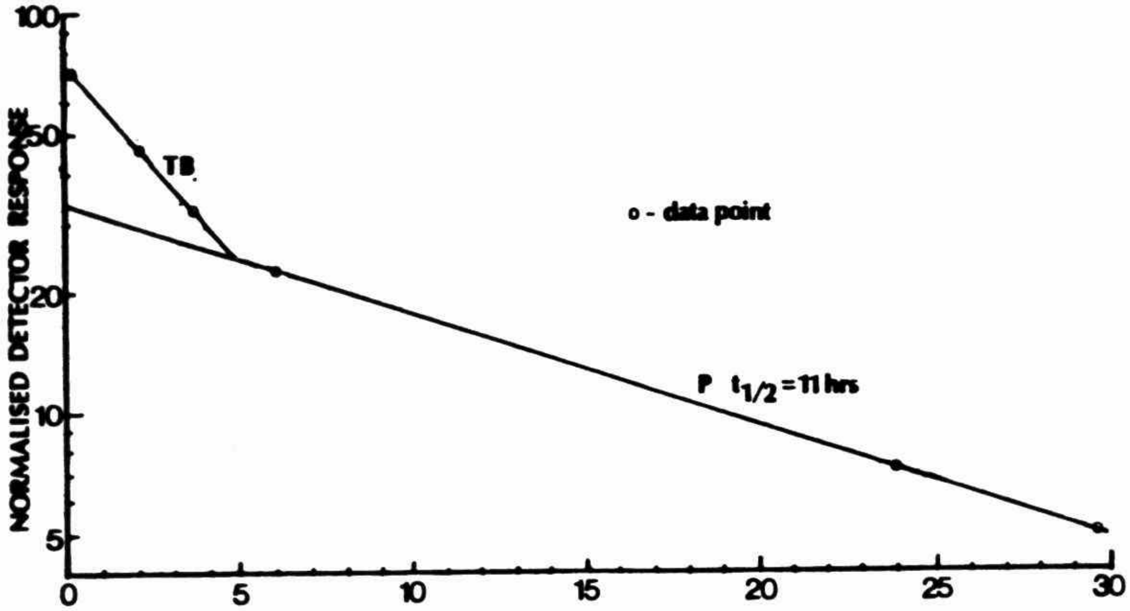

Fig. 2: Two stage chest clearance curve typically found in regional clearance experiments.

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# EXPERIMENTALLY DETERMINED MUTATION RATES AS AN AIR QUALITY STANDARD: EFFECT OF IN VIVO ADMINISTRATION OF CARCINOGENS ON MUTATION FREQUENCIES IN PULMONARY CELLS

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## ABSTRACT:

One health hazard arising from exposure to air pollution is respiratory tract cancer. The central role played by mutation in oncogenesis suggests detection of mutations induced in the cells at risk could provide a biological basis for establishing air quality standards. An assay to detect mutations arising in vivo in lung and tracheal epithelial cells is under development. To permit the use of cells which are difficult to clone, the assay utilizies autoradiographic detection of diphtheria toxin (DT)-resistant cells isolated from Chinese hamsters. Reproducable increases in the mutant frequency have been detected routinely, but these have been small, about three-fold, even though a potent lung carcinogen (urethane) and a potent mutagen (ethyl nitrosourea) were used. This contrasts with the 50-100 fold increases readily induced in Chinese hamster ovary cells by potent mutagens. Although mutation to DT-resistance can be readily induced in cultured Chinese hamster ovary (CHO) cells and the resistant cells detected autoradiographically, only small, approximately three-fold, but reproducible, increases in the frequency of DT-resistant pulmonary epithelial cells from animals exposed to a potent lung carcinogen, urethane, or a potent mutagen, ethylnitrosourea, have been detected.

In an effort to elucidate the reasons for the differences in response, we have turned to the use of another cell type, lung fibroblasts, and genetic marker, resistance to the purine analog 8-azaguanine. Treatment of Chinese hamsters with ethylmethanesulfonate or diethylnitrosamine results in a dramatic increase in the azaguanine-resistant fraction of isolated lung fibroblasts. In vitro treatment of these primary lung fibroblasts with ethylmethanesulfonate also induces large increases in the frequency DT-resistant cells. These results indicate that the failure of the autoradiographic assay to detect large increases in the frequency of DT-resistant pulmonary cells following in vivo exposure to mutagens is not due to factors such as ineffective in vivo dose levels, greater repair capacity in vivo, or a refractory genome. Work is in progress to determine if the in vivo results with DT-resistance is an artifact of the isolation procedure, as seems likely. If so, identification and elimination of it could lead to the desired increase in sensitvity of the autoradiographic assay. As an alternative approach, the use of a mutational assay using azaguanine-resistance in primary lung fibroblasts to detect pulmonmary carcinogens is also being explored.

## INTRODUCTION:

A quantitative assay for the toxic effects of airborne contaminants that is both relevant and inexpensive would be useful in establishing air quality standards. We are developing such an assay for carcinogenic air pollutants based on measurements of mutation in lung and tracheal cells in test animals. Most existing mutational assays are based on the ability of mutant cells to form colonies under selective conditions, but the difficulties in cloning pulmonary epithelial cells led us to consider alternate approaches, in particular the use of resistance to diphtheria toxin (DT) as a marker. Since DT exerts its toxic effect on cells by inhibiting protein synthesis (reviewed in Pappenheimer, 1977), individual cells which can incorporate radio-labelled amino acids into protein after exposure to DT can be identified by autoradiography. The feasibility of identifying DT-resistant cells by autoradiography has been demonstrated in vitro in cultured Chinese hamster ovary cells (Ronen et al., 1984) and human dipoid fibroblasts (Gupta and Singh, 1985), although the technique does not appear to be applicable to human lymphocytes (Tompkins et al, 1985).

We have previously reported the development of methods for the isolation and culture of epithelial cells of the lung and trachea of Chinese hamsters (Heddle et al., 1985). Although these culture conditions do not readily allow cell growth, especially of lung cells, cells can be maintained in culture for up to two weeks and are suitable for measurement of DT-resistance. Further, audioradiographic procedures have been adapted for use with these cultures. We report here that the effect of in vivo administration of mutagens and carcinogens on the frequency of DT-resistant lung and tracheal cells and on further development of an in vivo/in vitro somatic cell mutational assay utilizing pulmonary cells.

## MATERIALS AND METHODS:

The DT used in these studies was purchased from Connaught Laboratories (Toronto, Ontario). Urethane, ethylnitrosourea and azaguanine were obtained from Sigma Chemical Company (St. Louis, MO). Cell culture medium, trypsin and cell culture dishes were purchased from GIBCO Canada (Burlington, Ontario); fetal boving serum was purchased from BDH Chemicals (Toronto, Ontario). Tritiated leucine [L-(4,5-3H)-leucine, 1.92TBq/mmole] was obtained from Amersham Corporation (Oakville, Ontario) and NTB-2 emulsion and D-19 developer from Eastman Kodak Company (Brampton, Ontario).

The Chinese hamsters (5-6 weeks old when purchased from Cytogen Research and Development , Inc., West Roxbury, MA) used through out these studies were maintaned under the guidelines of the Canadian Council of Animal Care. Animals were housed in 20 degree rooms at 50% relative humidity in a 12-hour light-dark cycle. Hamsters were kept in wire cages with wood-chip bedding and given free access to food (Purina lab chow) and water.

Animals were treated with solutions of carcinogens (or vehicle alone) by intraperitoneal injection. After various times were allowed (see RESULTS) for in vivo expression of the mutant phenotype, animals were killed and the lung and/or tracheal epithelial cells removed and maintained in culture as previously described (Heddle et al., 1985). Lung cells from indivdual animals were used to determine the DT-resistant fraction, whereas it was necessary to pool tracheal cell from three animals to provide sufficient cells for the measurement. After 24 hours in culture to allow attachment to culture dishes, cells were treated with toxin. Various combinations of DT concentration and treatment time have been tested and are described below. After intoxication, cells were incubated with 3H-leucine and prepared for autoradiographic examination as previously described (Heddle et al., 1985)

In some studies, primary cultures of fibroblasts isolated from the pulmonary tissue of Chinese hamsters were employed to measure both DT-resistance (by autoradiography) and 8-azaguanine(AG)-resistance (by a clonal assay). The treatment of the animals, the isolation and culture of the fibroblasts, and the assay for AG-resistance were performed as described by Dean and Senner (1977).

## RESULTS AND DISCUSSION:

In order to determine whether treatment with carcinogens could increase the frequency of DT-resistant pulmonary epithelial cells, hamsters were treated with urethane (ethyl carbamate), known to induce lung tumors when administered to experimental animals by various routes, including intraperitoneal injection, to experimental animals (Shimkin and Stoner, 1975). As these experiments were intended as a demonstration of the feasibility of the approach, as well as to refine the selective conditions and provide preliminary information on the time required to express the DT-resistant phenotype in vivo, high doses of urethane were employed. Animals were treated with 1g/kg urethane daily for six successive days and sacrificed six days after the last injection. Lung and tracheal cells were treated with 100 LF/ml DT for up to 72 hours. The labelling indices (+ DT) of control animals, while considerably higher than found in CHO cells, are in good agreement with that reported previously (Heddle et al., 1985). At the most stringent selective conditionds employed, urethane produced only a three-fold increase in the DT-resistant fraction of tracheal cells and was even less effective in the lung (Table 1). Reducing the DT concentration to 1 or 10 LF/ml produced essentially the same result (Table 2).

**TABLE 1. EFFECT OF IN VIVO ADMINISTRATION OF URETHANE ON THE FREQUENCY OF DT-RESISTANT LUNG AND TRACEA CELLS**

| Time of Exposure to DT (h) | Frequency of DT-Resistant Cells (x $10^3$) | | |
|---|---|---|---|
| | Control | Urethane (6x)[1] | Urethane (3x)[2] |
| **Lung** | | | |
| 16 | 4.0 | 3.7 | 5.6 |
| 24 | 1.3 | 2.7 | 3.6 |
| 36 | 1.0 | 1.9 | 2.5 |
| 72 | 0.6 | 1.1 | 1.2 |
| **Trachea** | | | |
| 16 | 4.2 | 3.8 | 4.2 |
| 24 | 2.4 | 2.9 | 2.8 |
| 36 | 0.8 | 1.6 | 2.4 |
| 72 | 0.3 | 1.1 | 1.0 |

[1] Animals received 6 daily intrapertoneal injections of urethane (1g/kg) and were sacrificed 6 days after the last treatment.

[2] Animals received 3 injections of urethane at 3 day intervals and were sacrificed 3 days after the last treatment.

TABLE 2. EFFECT OF DT CONCENTRATION ON FREQUENCY OF DT-RESISTANT CELLS IN CONTROL AND URETHANE-TREATED ANIMALS

| Concentration of DT[1] (LF/ml) | Frequency of DT-Resistant Cells (x $10^3$)<br>Control | <br>Urethane (6x)[2] | <br>Urethane (3x)[2] |
|---|---|---|---|
| Lung | | | |
| 1 | 1.3 | 1.9 | 2.8 |
| 10 | 0.9 | 1.6 | 1.7 |
| 100 | 0.6 | 1.1 | 1.2 |
| Trachea | | | |
| 1 | 0.5 | 1.2 | 0.8 |
| 10 | 0.3 | 1.2 | 1.3 |
| 100 | 0.3 | 1.1 | 1.0 |

[1] Cells were incubated in DT for 72h.
[2] Animals were treated with urethane as described in Table 1.

As the efficacy of urethane as a lung carcinogen may not be entirely due to its mutagenic potency, animals were treated with N-ethyl-N-nitrosourea (ENU), an extremely potent mutagen in vitro or in vivo (cf. Natarajan etal., 1984; Russell et al., 1979). Hamsters were treated with 160 mg ENU/kg body weight and allowed 2-10 days expression time. The DT-resistant fraction of tracheal cells increased up to 6 days expression time, but again there was only a relatively small (approximately 4-fold) increase relative to control. Treatment with ENU did not affect the DT-resistance of lung epithelial cells (Table 3). Higher doses of ENU (up to 340 mg/kg, a dose which is fatal to 50% of the animals receiving it) were not effective

TABLE 3. TIME REQUIRED FOR IN VIVO EXPRESSION OF DT-RESISTANCE IN LUNG AND TRACHEAL CELLS

| Expression Time[2] (days) | Frequency of DT-Resistant Cells (x $10^3$)[1] | | | |
|---|---|---|---|---|
| | Lung | | Trachea | |
| | Control | ENU | Control | ENU |
| 2 | 0.5 | 0.6 | 0.2 | 0.5 |
| 4 | 0.5 | 0.6 | 0.2 | 0.7 |
| 6 | 0.6 | 0.5 | 0.3 | 1.3 |
| 8 | 0.5 | 0.8 | 0.3 | 1.3 |
| 10 | 0.5 | 0.9 | 0.3 | 1.5 |

[1] Isolated lung and tracheal cells were incubated with 100 LF/ml DT for 16h prior to labelling with $^3$H-leucine.

[2] Animals were treated by intraperitoneal injection with 160 mg ENU /kg body weight (or solvent alone) and sacrificed at the time indicated after treatment.

in producing furhter increases in the labelling index of tracheal cells, nor did they increase in the fraction of DT-resistant lung cells (Table 4).

If the high spontaneous frequency of cells which can incorporate tritiated leucine after DT treatment is not be due solely to the mutant fraction of those cells, increases in the frequency of DT-resitance due to mutation may be obscured. Attempts were made to improve the stringency of the selction by incorporating a brief (20

TABLE 4. EFFECT OF ENU DOSE ON THE FREQUENCY OF DT-RESISTANT LUNG AND TRACHEAL CELLS

| Dose ENU | Frequency of DT-Resistant Cells (x $10^3$) | |
|---|---|---|
| (mg/kg) | Lung | Trachea |
| 0 | 0.5 | 0.4 |
| 160 | 0.6 | 1.3 |
| 240 | 0.3 | 0.3 |
| 320 | 0.3 | 0.3 |

Animals were treated with ENV by intraperitoneal injection and allowed 6 days to express the mutant phenotype. Selection for DT-resistance was at 100 LF/ml DT for 72h.

minute) exposure to slightly acidic conditions (pH 4.4-6.8), which has been reported to enhance the cellular uptake of diphtheria toxin (Sandvig and Olsnes, 1980). Although exposure of cells to pH 6.0 buffer before intoxication did result in a substantial decline in the labelling index of cells from untreated animals, it also abolished the response induced by urethane (Table 5). Attempts have also been made in CHO cells to improve the stringency of the selection by incorporating exposure of the cells to toxin at temperatures which are not conducive to protein synthesis, but these attempts have not proven successful (data not shown). This method of increasing the responsiveness of the assay was thus abandoned.

TABLE 5. EFFECT OF pH ON THE LABELLING INDICES (+DT) OF LUNG AND TRACHEAL CELLS

| | Frequency of Labelled Cells ($\times 10^3$)[1] | |
|---|---|---|
| pH | Lung | Trachea |
| 4.4 | - | - |
| 4.8 | 1.5 | 0.2 |
| 5.2 | 2.6 | 0.2 |
| 5.6 | 3.8 | 0.7 |
| 6.0 | 1.2 | 0.4 |
| 6.4 | 0.9 | 0.4 |
| 6.8 | 1.1 | 0.3 |
| 7.2 | 4.5 | 0.6 |
| 7.4 | 4.8 | 0.7 |

[1] Cells were maintained in culture 24h after isolation, then exposed to buffer at the indicated pH. The buffer solution was removed and centrifuged to collect any cells which may have detached. These cells were returned to the dishes in medium containing 100 LF/ml D+ and incubation continued for 72h.

Although there were indications that mutations leading to DT-resistance could be detected by an autoradiaographic assay with tracheal epithelial cells, at least, the sensitivity of the procedure was not sufficient to permit its proposed use in establishing air quality standards. Many possible explanations could be devised for the observed findings, and, to help establish which of these may be correct, we turned to the use of another genetic marker, resistance to 8-azaguanine. Dividing cells are required for this procedure so subsequent studies were performed on primary lung fibroblasts isolated from Chinese hamsters.

We first asked whether mutations can be induced in somatic pulmonary) cells in vivo by known carcinogens and mutagens. In agreement with the results of Dean and Senner (1977), treatment of Chinese hamsters with ethylmethansulfonate (EMS) or N-nitrosodiethylamine (DEN). was found to produce a dose-related increase in the AG-resistant fraction of primary lung fibroblasts (Table 6). Although the AG-resistant mutants were induced in fibroblasts and the DT-resistant mutants were induced in epithelial cells, these two cell types are closely apposed in the lung. Thus these results indicate the lack of response observed with epithelial cells is probably not a result of failure of the test compounds to reach pulmonary tissues in sufficiently high concentration to produce an effect, nor does it appear that the discrepancy in results obtained with the autoradiographic assay in CHO and pulmonary epithelial cells can be attributed to more efficient repair of mutagenic lesions in vivo or to a generally lower induced mutation rate in vivo than in established cell lines. It could be, however, that fibroblasts used in the clonal assay differ in these particulars from epitheial cells used in the autoradiographic assay.

We then asked if the failure to detect mutation to DT-resistance in vivo was a consequence of the genetic character of the cells exposed to toxin. It could be that hemizygosity of CHO cells allows

TABLE 6. EFFECT OF IN VIVO ADMINISTRATION OF EMS AND DEN ON THE FREQUENCY OF 8AG-RESISTANT PRIMARY LUNG FIBROBLASTS

| Dose<br>(mg/kg) | Mutant Frequency[1]<br>(x $10^4$) |
|---|---|
| **EMS** | |
| 0 | < 0.3 |
| 50 | < 0.4 |
| 100 | 1.1 |
| 200 | 2.4 |
| 400 | 6.8 |
| **DEN** | |
| 0 | 0.1 |
| 250 | 2.6 |
| 500 | 3.9 |
| 1000 | 6.1 |

[1] Animals were treated with EMS and DEN but intraperitoneal injection; after treatment fibroblasts were isolated 5h after treatment and the 8AG-resistant fraction determined 5h later, according to the method of Dean and Senner (1977).

recessive mutations to be detected that would not be evident in diploid cells. Accordingly, freshly isolated lung fibroblasts were treated in vitro with EMS, and the DT-resistant fraction determined following five days in culture. A marked increase in the mutant fraction over control was obtained (Table 7), indicating the

TABLE 7. EFFECT OF IN VITRO ADMINISTRATION OF EMS ON THE FREQUENCY OF DT-RESISTANT PRIMARY LUNG FIBROBLASTS

| Time[1] | Frequency of DT-Resistant Cells (x $10^6$) | |
|---|---|---|
| (days) | Control | EMS[2] |
| 4 | 9.2 | 240 |
| 7 | 2.6 | 210 |
| 14 | 0.6 | 200 |

[1] Fibroblasts were isolated and maintained in culture for the indicated time prior to treatment with EMS.

[2] Cells were treated with 250 ug/ml EMS for 20h and then maintained in culture for 5 days before exposure to 100 LF/ml DT for 16 hours. Resistant cells were detected autoradiographically.

autoradiographic assay is suitable for use with hamster diploid cells (as it is for diploid human fibroblasts; Gupta and Singh, 1985). In one experiment, however, ultraviolet irradiation of primarly lung cells did not increase the labelling index of fibroblasts in the presence of DT (data not shown).

Preliminary results indicate that treatment of Chinese hamsters with EMS does not appreciably increase the DT-resistant fraction of lung fibroblasts isolated from the animals soon after treatment and allowed to express the mutant phenotyupe in vitro for five days, although 8-azaguanine-resistance is increased in another cohort of the same cell population. If these results are confirmed, it will suggest that the failure of to detect DT-resistant fibroblasts, and possibly epithelial cells as well, following in vivo administration of mutagens, is an artifact arising from the isolation procedure. Alternatively, there may be differences in the response of fibroblastic and epithelial cells to mutagens in vivo. This possibility is under investigation using a clonal assay for azagunanine-resistant tracheal cells to compare their response to that of pulmonary fibroblasts.

## CONCLUSIONS

Optimal conditions for detecting DT-resistant epithelial cells of the lung and trachea have not yet been found, but recent findings suggest that the problems encountered may arise as an artifact of cell isolation and are not a failure to induce mutations in the target cells. The ease with which AG-resitant lung fibroblast can be detected offers another approach to detect potential pulmonmary carcinogens, which is also being actively pursued.

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# THE HAMILTON STUDY: EFFECT OF FINE PARTICLES ON THE RESPIRATORY HEALTH OF SCHOOL CHILDREN

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## BACKGROUND

Early studies from our laboratory showed that the persistent aerosol in the atmosphere over Hamilton could be visualized and its extent and altitude measured by a simple technique using a searchlight (1). In this study we demonstrated that aerosols were frequently trapped in inversion layers over the city, especially when the wind came from the northeast over the cold lake.

We also operated a pollution sampling network between Lake Erie and Hamilton using Hi-Vol samplers for "Total Suspended Particulate" (TSP) and Andersen cascade impactors to measure particle size distribution (PSD). We were able to show that both the concentration and size of the particles moving from Lake Erie in the direction of the prevailing wind toward Hamilton increased substantially. The greatest increase began at the city limits, and substantial gradients were observed while moving in a northeasterly direction over the industrial area (2).

Thus before we started the present study, we had good evidence that there were substantial gradients of TSP and PSD the highest levels being more than twice the lowest levels. As a consequence, we decided to make use of these gradients to carry out an epidemiological study of the effects of air pollution on respiratory health.

## METHOD

We decided to carry out our study by examining the effects of air pollution and other environmental factors on the respiratory health of a sample of elementary school children in the city of Hamilton. We were aware that many factors other than air pollution influenced respiratory health, so our study was set up to examine the relative contribution of many environmental factors on the respiratory health of our school children.

We chose to study children enrolled in schools of the Board of Education of the City of Hamilton in grades 2, 3 and 4 who had not reached their 11th birthday by December 31, 1978. There were over 8400 children in these grades, and sample size calculations indicated that we would require 3200 in a stratified random sample (5). An attempt was made to have the children uniformly distributed over 4 "quadrants" of the city (west upper - WU, east upper - EU, west lower - WL, and east lower - EL), as shown in Figure 1. Figure 1A shows the areas of the city, and the locations of the aerosol samplers. Figure 1B shows the distribution of the schools with respect to the samplers. In the second year (Year II) of the study (reported here), which took place in 1980, we added children of the same age as the original cohort from three schools in the industrial core (IC) of the city, a subsection of the EL quadrant. The total number of children studied in Year II was 3588. Parents were interviewed and children studied once each year for a total of three times, essentially in the calendar years 1979, 1980, and 1981. Further details of the sample are given in a previous report (5).

## Measurements of Suspended Particles

For the purposes of the epidemiological study, we set up a pollution sampling network to measure TSP at 27 sites and PSD at 7-9 sites. At the time we began the study, the Ontario Ministry of the Environment (OME) operated 11 TSP sites in an abatement-oriented network in the industrial and commercial parts of the city, and had no ongoing measurements of particle size distribution. Since for our purposes we had need of an estimate of an exposure which was more population based, we found it necessary to complement the OME network with 16 TSP sites of our own, and to set up a network of PSD samplers (7 in 1979 and 1980, 9 in 1981). Our TSP sites were for the most part located in residential areas, but the PSD locations were distributed across the city.

TSP was measured with standard Hi-Vol samplers (General Metal Works GMWL-2000H) using glass fibre filter paper, and PSD was measured using the same housing, but with a 4-stage cascade impactor designed to operate at 20 cfm (Andersen 2000) (4). Flow recorders were used on all TSP samplers. PSD flow was set to 20 cfm before sampling by means of variable auto transformers on all PSD samplers, and was measured and recorded after sampling. Filters were conditioned at known humidity before and after exposure, and weighed with a Mettler Gram-matic balance. Both networks were operated every six days synchronously with the North American Synoptic Six-day Cycle. Quality control procedures were used, which will be reported elsewhere.

## Summarization of Data

For TSP, the results for each site were expressed in ug/m$^3$, and this was recorded together with the site identification number and the date of sampling. The five or six values for a given site were used to generate a monthly geometric mean value for that site. If fewer than three values were available for a site for a given month, the data for that month were declared "missing". This criterion also held for PSD. In order to summarize the results of the measurements obtained by the PSD network, we chose to obtain monthly average values in the following way. Since the sampler collected aerosol on four separate impactor stages as well as on a backup filter, on a given sampling day we could measure it in each of five size ranges, and this was expressed in micrograms per cubic meter. To obtain a summary of information for a month, the loadings on corresponding filters were added together for the five or six sample days, and a mean monthly loading for each stage or filter was calculated. From the five monthly loadings, aerodynamic mass median diameter could be calculated in the standard manner and expressed as MMD in micrometers (um) (4). In addition, we decided to add the loadings of the first two stages together including particles greater than 3.3 um, and referred to this as the "coarse fraction" (CF). We added the loadings of the third and fourth stage as well as the backup filter together and referred to this as the fine fraction (FF) (i.e. that less than 3.3 um). Thus for a given month, we had a measure of MMD obtained from all the loadings for that month, and a measure of coarse and fine fraction which was obtained from the sum of the corresponding loadings for that month, and expressed as $ug/m^3$.

## Analysis of Data from Air Pollution Networks

Our objective was to determine an exposure which was as closely as possible unique to each child in the study. For practical purposes we decided to use a time resolution of one month, and a spatial resolution determined by the location of the school the child attended at the time the child was tested. Thus we had need of a method by which we could estimate a monthly exposure on a school-by-school basis.

Using the monthly values obtained for each site for each variable (TSP, MMD, CF, FF), a response surface was calculated which expressed in three-dimensional terms (vertical direction -

aerosol variable; horizontal "x" - easting geographical co-ordinate; horizontal "y" - northing geographical co-ordinate) an estimate of the distribution of the aerosol as a function of the geographical plane of the city. For MMD, CF and FF the form of the response surface model was:

$$MMD = B0 + B1(E) + B2(N) + B12(EXN) + e$$

where MMD is the response value,

B0, B1, B2, and B12 are regression coefficients,

E and N are normalized easting and northing geographical coordinates, and

e is the error term

In addition, the coefficient of determination of the surface could be calculated, indicating the proportion of the variation in the data explained by the model we fitted. In most cases over 2/3 of the variability in the data was accounted for by the fitted model.

To calculate an exposure value for an individual child, an annual mean was determined by taking the arithmetic mean of monthly values of a given variable for the school location at the time of testing: for the month of testing, the 9 months preceding it, and the 2 months following it. The yearly average was used because we were looking for long-term or chronic effects both in respiratory symptoms and as indicators of these in pulmonary function, and because plots of yearly air quality measurements indicated that seasonal cycles repeated within the years.

### Respiratory Health

The respiratory health of the children, and home environmental and socio-economic variables were assessed by means of a questionnaire administered to the children's parents. Pulmonary function tests were conducted in the schools after having received consent from the parents of the children. Interviews and tests were conducted once a year for a period of three years.

## RESULTS

### Suspended Particles

Figure 2 shows contours of mass median diameter obtained using the response surface model for the month of May 1980. Each small number which defines the contour line represents twice the mass median diameter defined by that line; i.e., "8" = 4 um MMD. The particle size to which the children were exposed was calculated on the basis of monthly maps of this type.

We found that the pattern of the distribution of particle size and fine and coarse fraction was similar from year to year, as is shown in Figures 3 and 4. Figure 3 shows the calendar yearly response surface contours for the three years 1979, 1980 and 1981. Although the nature of the contours are determined in part by the model, it is apparent that the shapes of the contours are similar from year to year. The southwest-northeast axis of symmetry probably relates to the two dominant wind directions: southwest (35% of the time) and northeast (11% of the time) (4). It is clear from these contours that a substantial gradient exists over the city. In Figure 4, the numbers on each contour represent tens of micrograms per cubic meter. For the years 1979 and 1980 the patterns are quite stable, with substantial gradients in both fractions. In 1981 however, when a recession and a prolonged steel strike reduced industrial activity, the reduction in the gradient for the coarse fraction was much more marked than that seen for the fine fraction.

## Respiratory Health

In the year of the study being reported here (Year II, Jan. - Dec. 1980), a total of 3439 children had both a completed parental interview and a pulmonary function test session. The response rate for interviews was greater than 95% in all years, and the response rate for testing was greater than 92% for Years II and III, and greater than 89% in Year I.

Details of the prevalence rates for respiratory symptoms and the values of pulmonary function will be reported elsewhere. In this report, for the purposes of associating with the effects of particle size we have selected two indicators of respiratory health (cough, and episodes of bronchitis), and two indicators of pulmonary function, both adjusted for body size.

Several factors have previously been implicated in a reduction of respiratory health of children even without an effect of pollution. We have previously reported on this problem and the extent to which it might have influenced our data, and for that reason found it necessary to use a method of analysis which was more complex than the demonstration of simple independent associations (i.e. not adjusted for confounding factors) between health effects and air pollution (5).

## Relative Strength of Environmental Effects

We chose to analyse these interdependent associations using stepwise multiple regression techniques. Initially we found a variety of independent variables that had shown at least one significant association with health-related outcomes. A list of these independent variables is given in Table I. We found, for example, that when examined as a single variable, gas cooking was associated with an increased prevalence of colds going to the chest. In the multiple regression analysis, all variables are introduced together and the computer selects the variables with the strongest and most significant effects, rejecting others that are highly correlated with the one selected. For continuous dependent variables, multiple linear regression was used; for binary dependent variables such as symptoms, the multiple logistic regression was used. A variable was said to be significant if the probability of a chance association was less than 0.05 ( =0.05).

## Symptoms

The results of the logistic regression for symptoms are shown in Table II. The logistic regression calculates the influence of an independent variable on the probability of occurrence of an outcome variable according to the function shown in the Table. For example, two independent variables that were found to be significantly associated with the probability of a child having a cough during the day or night were: maternal cough, and hospitalization as an infant with respiratory disease. All other factors included were not significantly associated. In the table, the "p" value relates to the probability of the association being by chance (not the probability of the cough). Also in the table it can be seen that the probability of a child having an episode of cough and phlegm lasting at least three weeks (bronchitis) was significantly associated with three factors, maternal smoking being added to the other two.

## Pulmonary Function

One of the measurements frequently made in respiratory epidemiological surveys is that of Peak Expiratory Flow (PF). Since the magnitude of PF is dependent on the size of the child, we have adjusted the values to the mean height of all the children (APF). In the example shown in Table III, the first equation shows that APF is reduced with an increased magnitude of the fine fraction. In addition, if the child shares a bedroom APF would be further reduced. Only these two variables were found to have an effect on adjusted peak flow. The MEF 75 is also a function of body size and must be suitably adjusted. From Table III, it may be seen that AMEF75 is significantly affected by three factors: maternal smoking; hospitalization of the child as an infant; and increased particle size. The observation with respect to particle size (MMD) should probably be interpreted in another way, however. It would be more logical to expect a reduction in function associated with particle exposure, and thus it could equally be said that MEF75 is reduced with a reduction in particle size, which would be consistent with the findings for peak flow.

## DISCUSSION

We have carried out a study of the effects of ambient aerosol pollution and other environmental factors on the respiratory health of over 3500 school children in an industrial city. We have demonstrated a substantial gradient in aerosol concentration and aerodynamic mass median diameter over the area in which the children live and attend school. By taking advantage of this gradient in exposure, and by assessing a variety of independent variables, we have shown that there are strong effects of the home environment, particularly of maternal smoking, and significant consequences of severe respiratory disease in infancy (which has been shown by others to be associated with smoking in the home) (6). We have also demonstrated effects of the fine fraction of aerosol pollution. Although this is based on a cross-sectional analysis of one year of data from the study, it is new evidence that the fine fraction of urban aerosol is associated with a degradation of pulmonary function in school children. There have been many studies of the effects of air pollution on the respiratory health of children (7-16), but only one has measured (but not yet reported) the effects of particle size (17).

We have previously reported that indices of the Forced Vital Capacity (FVC) have greater discriminatory power in separating children with symptoms from those without, compared to tests using nitrogen washout (18). For the purposes of this report we have included the results of measurements of two pulmonary function variables (PF and MEF75) adjusted for height. We also examined other indices of the FVC, such as $FEV_1/FVC$, and height-adjusted $FEV_1$, MMEF, and MEF50. Three tests ($FEV_1/FVC$, MMEF, and MEF 50) all showed an effect of early hospitalization, and maternal smoking. Two showed effects of particles, which were just significant at the 0.05 level; MMEF showed an association with TSP, and $FEV_1$ an association with MMD. Both associations were positive, suggesting the interpretation of a "fine particle effect", as we have evidence of a positive association between TSP and MMD. Thus the results of the other pulmonary function tests which we carried out are similar to those we presented in more detail, but the strength of the associations were less.

The same general points can be made about respiratory symptoms. The other symptoms that were examined, such as presence of any cough (not necessarily chronic), persistent wheeze, or colds "going to the chest" were all associated with hospitalization as an infant and maternal cough or maternal smoking. "Cold going to the chest" was associated with all 3 of the above, as well as concentration of TSP above the median (50 $ug/m^3$), but only at the 5% level. Symptoms were not associated with our measures of particle size.

## SUMMARY

We have operated a network of Andersen 2000 cascade impactors in the industrial city of Hamilton, Ontario, Canada, and have demonstrated statistically significant gradients in particle size over the city. We have carried out at the same time in the same city an epidemiological study of respiratory health of over 3500 elementary school children in whom we have measured pulmonary function and the prevalence of symptoms, as well as characteristics of the home environment, including family smoking. We have developed a method of determining exposure of each child to particles which depends on the location of the child and the characteristics of the gradient we have observed. We found strong negative health effects of the home environment, particularly maternal smoking, and relatively weak effects of the ambient air quality. We did observe, however that there was a statistically significant association between decrement in pulmonary function and an exposure to fine particles.

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Table I. Independent Variables in Regression

- Hospitalization at < 2 Yr. of age for Respiratory Illness
- Mother Has Cough
- Mother Smokes / # of Cigs/Day
- Father Smokes / # of Cigs/Day
- Father Has Cough
- Family Income
- Father's Occupation
- Mother's Occupation
- Gas Cooking
- Child Shares Bedroom
- Family: # Cigs/Day
- Total Suspended Particulates
- Mass Median Diameter
- Coarse Fraction
- Fine Fraction

Table II. Respiratory Health Logistic Regression

Function: P = 1 / (1 + e * * -[A + B1X1 + B2X2 + ...])

| SYMPTOM | COEFF | COEFF VALUE | VARIABLE | SIGNIF OF COEFF | CODE | | |
|---|---|---|---|---|---|---|---|
| | | | | | Pres | , | Abs |
| Cough Day Or Night | A | -1.80 | CONSTANT | (p<0.00001) | --- | | --- |
| | B1 | 0.44 | MACOF | (p<0.00001) | 1 | , | 0 |
| | B2 | -0.34 | HOSP<2yr | (p<0.001) | 1 | , | 2 |
| Episode of Cough And Phlegm >3 Weeks | A | -1.69 | CONSTANT | (p<0.00001) | --- | , | --- |
| | B1 | 0.35 | MACOF | (p<0.00001) | 1 | , | 0 |
| | B2 | -0.29 | HOSP<2yr | (p<0.001) | 1 | , | 2 |
| | B3 | 0.19 | MASMOKE | (p<0.003) | 1 | , | 0 |

Table III: Pulmonary Function Stepwise Multiple Linear Regression

Function: PULM. FUNC. = A + B1X1 + B2X2 + ...

| PULMONARY FUNCTION VARIABLE | COEFF | COEFF VALUE | VARIABLE | SIGNIF OF COEFF | UNITS OR CODE | |
|---|---|---|---|---|---|---|
| | | | | | Pres , | Abs |
| Height-Adjusted Peak Flow | A | 4.220 | CONSTANT | ($p<0.00001$) | --- | --- |
| | B1 | -0.010 | FF | ($p<0.00001$) | $ug/m^3$ | |
| | B2 | -0.049 | SHAROOM | ($p<0.04$) | 1 , | 0 |
| Height-Adjusted MEF 75 | A | 0.544 | CONSTANT | ($p<0.0001$) | --- | --- |
| | B1 | 0.125 | MMD | ($p=0.0001$) | um | |
| | B2 | -0.002 | MACIG | ($p=0.0001$) | Cigs/Day | |
| | B3 | 0.067 | HOSP<2yr | ($p<0.02$) | 1 , | 2 |

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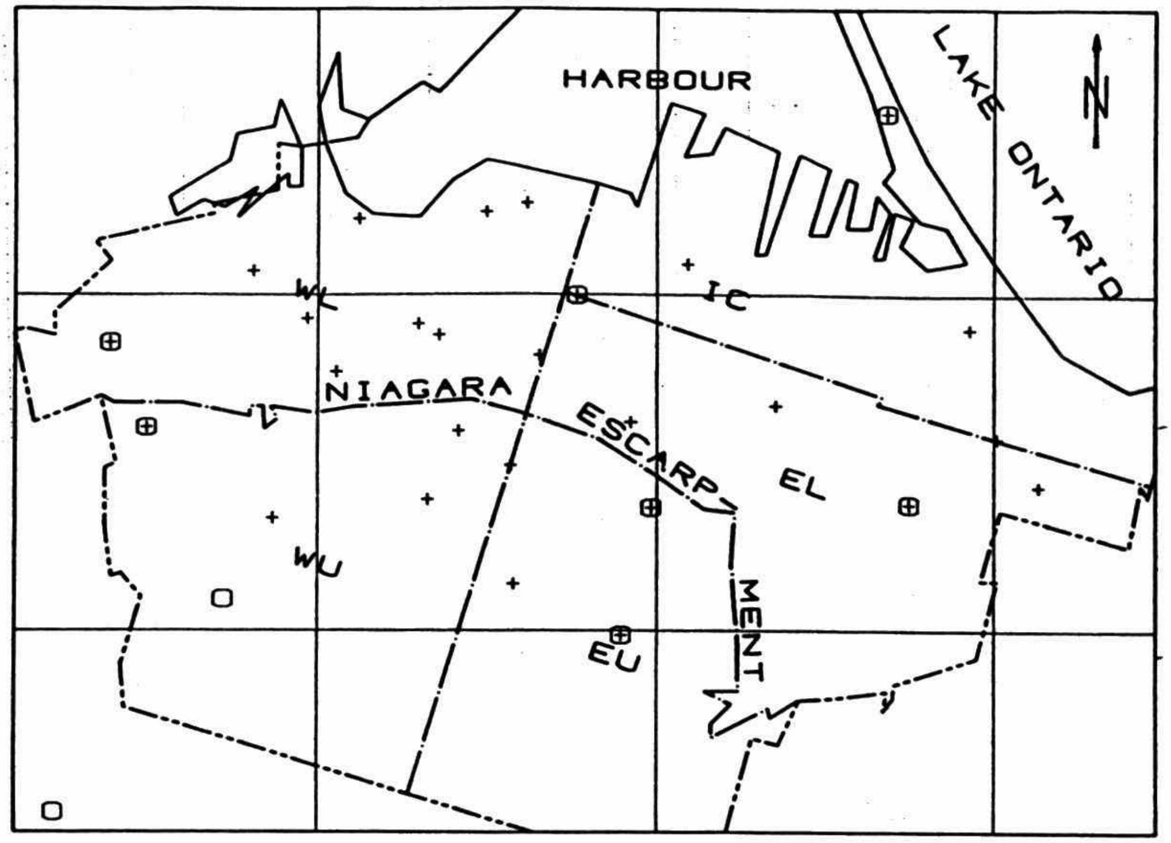
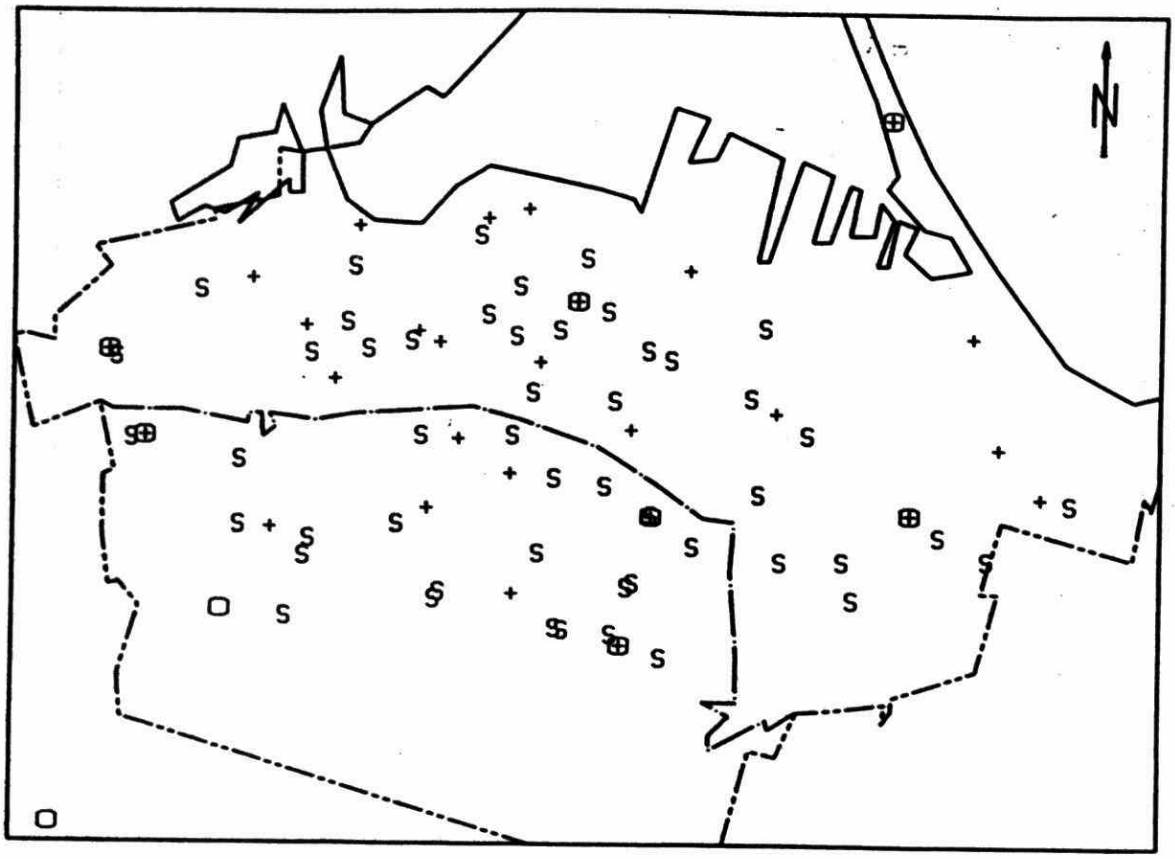


Figure 1 (A) Outline map of Hamilton, Ontario. City limits shown as broken line with two dashes; study area boundaries by broken line with dots. Grid lines are Universal Transverse Mercator at 5000 m intervals. Grid reference for the intersection point at "WL" is 590000 E, 4790000 N (Zone 17T). Crosses are TSP sites; open figures are PSD sites; a number of PSD sites are located at TSP sites.



(B) Same area as (A), but showing locations of schools ("S") as well as sampling sites

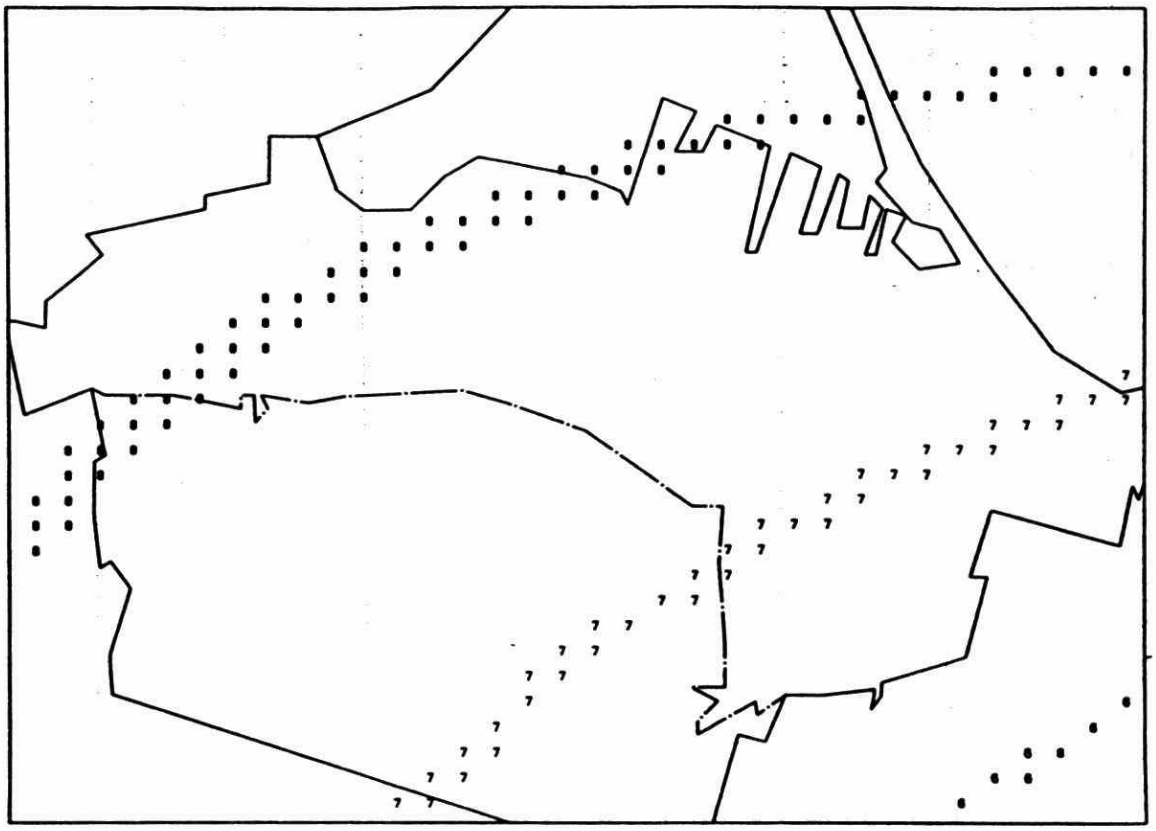
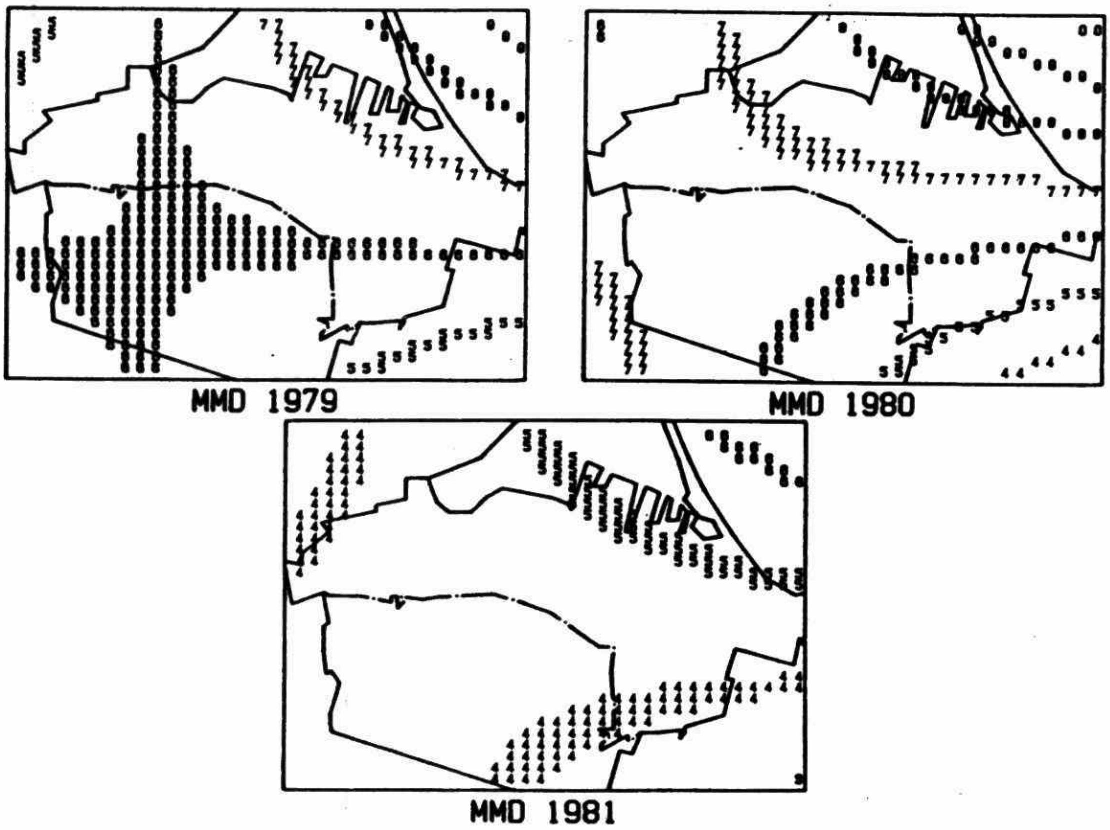


Figure 2 Distribution of particle size over the City of Hamilton for May, 1980. Contours of MMD derived from the response surface. Mass median diameter in micrometers may be obtained from the small numbers defining the isopleth by dividing by 2.

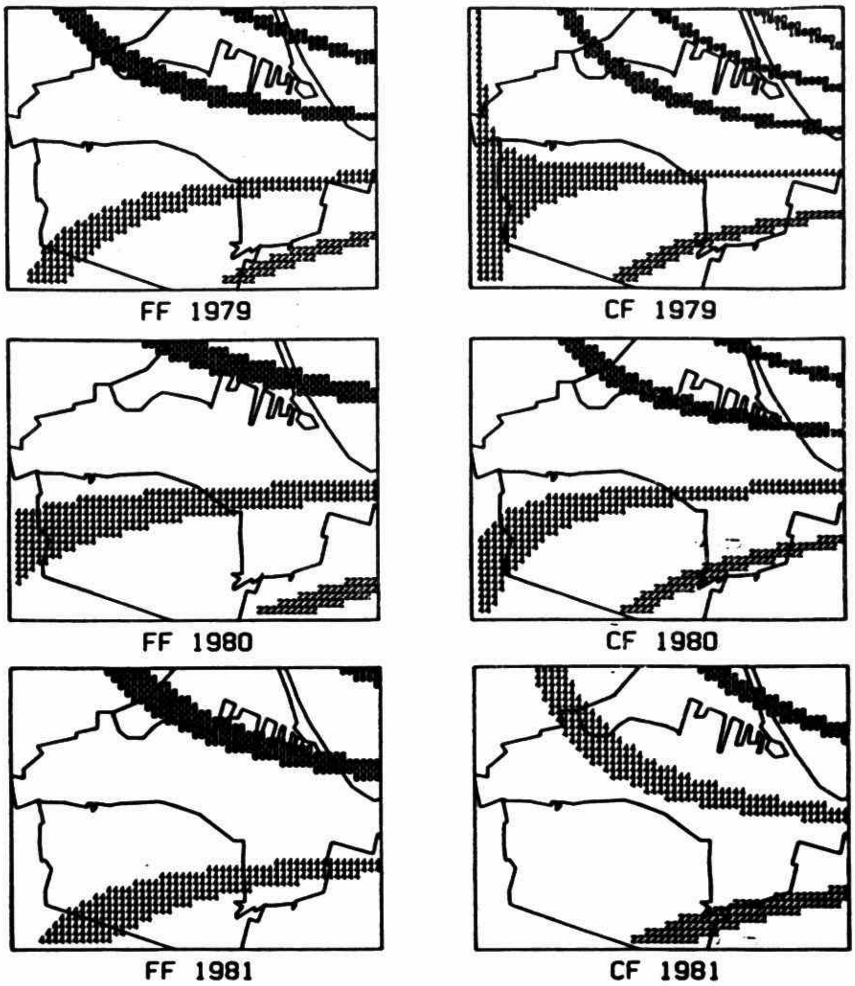


MMD 1979

MMD 1980

MMD 1981

Figure 3 Distribution of particle size over Hamilton: averages for three years. Contours derived from the response surface for yearly (12-month arithmetic mean) of MMD; isopleth system as in Figure 2.



FF 1979

CF 1979

FF 1980

CF 1980

FF 1981

CF 1981

Figure 4 Distribution of fine fraction: FF, (<3.3 um) and coarse fraction: CF, (>3.3 um) of particles over Hamilton for 3 years. Concentration defined by the isopleths in $ug/m^3$ are obtained by multiplying isopleth number by 10.

# BIOLOGICAL MONITORING OF ENVIRONMENTAL GENOTOXICITY

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# BIOLOGICAL MONITORING OF ENVIRONMENTAL GENOTOXICITY

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## Abstract

The SISTER CHROMATID EXCHANGE (SCE) assay has been modified so that wild mice can routinely be used to monitor the environment for general genotoxicity. The use of bromodeoxyuridine tablets and improvement of the staining procedure have permitted the processing of an adequate-sized sample (30 mice or more) at one time with consistently good results. This procedure has now been applied to wild mice collected from a number of localities in southwestern Ontario over the last three years. The annual temporal and geographic patterns have been similar from year to year. The SCE values/mouse are low in the early spring (prior to the spraying of crops with pesticides), high in the late spring (spraying period) and generally low in the summer (post-spraying period). The geographic pattern involves high SCE values in mice captured in the western part of the study area (Essex County) and lower values in those from the eastern section (Kent and Elgin Counties). Also we have examined the effects of a number of factors on SCE levels and their variability. These have included: short-term stress, age, genetic heterogeneity, some commonly used herbicides, several chemicals encountered in the plastics-molding industry and fumes in a diesel garage. Only chromic acid (plastics-molding industry) and diesel fumes significantly increased SCE values.

The MICRONUCLEUS ASSAY OF PERIPHERAL BLOOD was selected as a back-up system to permit corroboration of the SCE results. This assay was chosen because: 1) with fluorescence microscopy the typing of micronucleated cells is very straightforward and can be done rapidly, 2) the mouse is not sacrificed and blood samples from the same animal can be obtained at different times after treatment, 3) no pretreatment of animals is necessary prior to obtaining testable blood samples, and 4) a single recent (within 72 hours) exposure of an animal to a genotoxic agent can be distinguished from both an earlier exposure and chronic exposure. Furthermore, although most agents induce both SCEs and micronuclei there are some that cause the latter but not the former making the two tests complementary. So far a time-response curve has been developed for two compounds, cyclophosphamide and mitomycin C. Peak induction of micronuclei occurs at around 48 hours after injection of either agent. This appears to be about 12 hours later than induction of micronuclei in bone marrow cells. The micronucleus assay has been applied to wild mice being examined for SCEs as well as mice being used in several experimental situations.

The results of both tests will be presented, compared and discussed in detail.

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# BIOLOGICAL MONITORING OF ENVIRONMENTAL GENOTOXICITY

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## INTRODUCTION

Many of the studies being presented at this conference are concerned with the chemical and physical detection and analyses of pollutants. Such studies are essential, especially, since man has been extremely successful in saturating his environment with a plethora of agents which affect the quality of that environment and even his health. In considering the effects on man, we, however, frequently ignore the fact that the pollution problem is multifaceted, that many factors are interrelated and interwoven to give an end result and that physical measurement may not be relevant. It is, therefore, important that assessments of the effect of environmental pollution be done under biologically realistic conditions. This generally implies in situ monitoring. Although such monitoring may simply involve the measuring of toxic agents which have accumulated in organisms or the physiological effects of such agents in specific tissues, it is the short and long term genetic effects that generally are of most interest. Short term genetic damage may be reflected in the development of cancer or tumors, whereas the long term effects include the accumulation of deleterious gene mutations which are passed on from generation to generation.

In situ monitoring permits concentrating, under a set of natural conditions, on the effects of a single pollutant or occasionally a group of pollutants. It also permits general monitoring of the environment where the genotoxicants have not been specifically identified. In both situations a number of other factors enter the picture. These can be chemical, physical and even biological. Such factors may have either a promotional or an inhibitory effect on the agent(s) being monitored. In some instances, synergistic interaction may be encountered involving agents that alone, at best, would have very subtle effects. Furthermore, sometimes even nonmutagenic compounds may act synergistically with known mutagens.

Conversely, the use of laboratory strains of organisms, under laboratory conditions, could result in an overestimate of the genetic damage that is being caused by a certain agent or group of agents because wild (non-laboratory) organisms may tend to be more resistance to the agent(s) as a result of mutation and natural selection. Such resistance could, for example, be the result of the evolution of new detoxification steps, the lack of sensitivity to a particular agent, improved DNA repair efficiency.

*In situ* monitoring also provides data on temporal variations (seasonal, diurnal, biorhythmic) and geographic patterns. The latter may reflect the establishment of new industries, landfill sites, nuclear power plants, farming practices.

The SISTER CHROMATID EXCHANGE (SCE) TECHNIQUE in mice has been proposed as a sensitive, *in situ* monitoring system which can provide us with an EARLY WARNING ABOUT CHANGES IN GENOTOXIC LEVELS in the environment. The procedure which was described at this Conference several years ago has been modified so that it consistently gives good results with a minimum of technician effort. In the past eighteen months we have continued examining natural populations of the house mouse, *Mus domesticus*, for SCE levels in order to reinforce the 1983 and 1984 findings. In the process we have also developed a data set of currently "typical" SCE values against which future values may be compared. Such a data base should be especially valuable if and when the Detroit, Michigan incinerator comes on line or other pollution crises surface. Since seasonal fluctuations in SCEs have been observed and since we postulate these fluctuations to be associated with agricultural spraying, we have examined several herbicides that are commonly used in southwestern Ontario. In looking for a 'crisis' situation, that is one where genotoxic levels are high, we keyed on three industrial situations which we thought might be good candidates (a chrome-plating plant, a plastics processing shop and a diesel-repair garage). We have also been concerned about the reliability of the SCE technique when applied to wild mice. These include effects resulting from: a) stress encountered in trapping, transporting and general handling of the wild mice, b) the high genetic variability characteristic of wild mice, c) possible differences between inbred and wild mice in their response to mutagenic agents. We have also been concerned with improving the sensitivity of the SCE assay and to this effect we have been looking at several tumor/carcinoma/mutation promoting and inhibiting agents. Finally, we have spent considerable effort applying (and to a limited degree modifying) a second procedure, the ASSAYING OF MICRONUCLEI IN PERIPHERAL BLOOD of mice, so that we can substantiate the results of the SCE technique.

## SCE ANALYSIS

In SCE studies, newly formed DNA strands are labelled with 5-bromo-2'-deoxyuridine (BrdU). This, after several chromosomal replications results in differential staining of two sister chromatids. Differential staining permits the detection of exchanges between such chromatids.

The introduction of BrdU was modified to permit the processing of a large number of mice within hours after being caught. This was done by switching from serial injections to implanting tablets. Each tablet was formed by compressing 50mg of BrdU. A tablet was then partly coated by melted paraffin, and inserted subcutaneously on the dorsum of anesthetized mice. Twenty-four hours later 4ug/g of colchicine were injected intraperitoneally and three hours after that the mice were sacrificed by cervical dislocation.

The rest of the procedure was also streamlined. Femurs were removed and bone marrow cells were flushed into a warm (37C) hypotonic KCl solution (0.075M). The cells then were put through two stages of fixation in a cold glacial acetic acid/methanol mixture. On fixation, the cell suspension was applied dropwise to slides and air dried. After a 24 hour aging period, the slides were incubated in a fluorescent stain, Hoechst 33258 (10ug/g of distilled water), for 15 minutes. The slides were then placed in a staining jar containing Sorensen's buffer (pH 8.0), covered with saran wrap and exposed to a bank of four fluorescent tubes at a distance of 15cm for approximately 23 hours. At the end of this period a single slide was removed and stained with Fisher's Giemsa for 8 minutes. If no differential staining was seen the rest of the slides were exposed to the fluorescent lights for another 10 minutes and another test slide was examined. This was repeated until satisfactory differential staining was observed and then all the remaining slides were stained.

The above procedure eliminates serial injections of BrdU and handling of slides individually until the scoring is to be done. It also permits the staining of large numbers of slides at one time, since test slides insure that no run is lost because of poor differential staining of the chromatids. This procedure is highly suitable for survey work because up to 30 mice can be sacrificed and processed relatively easily at one time. This permits the processing in a single run a large sample and an adequate-sized control group. Scoring can be done later.

## CONTINUED MONITORING OF NATURAL MOUSE POPULATIONS FOR SCEs

The monitoring of natural populations of mice for sister chromatid exchanges begun in 1983 was continued into 1986. Again the mice were collected from corn cribs as they were being emptied. The SCE values of the mice collected in 1985 and 1986 are presented in Table 1. For comparison a summary of the 1985 and 1986 data are given together with those of the previous two years in Table 2.

In both 1985 and 1986 the Essex County samples showed seasonal peaks in June and July. This corresponds to the period when crops were being sprayed with herbicides. Unfortunately because no mice were obtained from Kent County during the prespraying season (farmers were not emptying cribs at this time) only Essex County samples can be examined for seasonal patterns. These patterns are consistent with those seen previously. The 1986 results from Kent County are unique, in this regard, because they show no seasonal patterns. Perhaps this is due to the small sample sizes obtained in May. Unlike the 1984 results, the sexes in the 1985 and 1986 samples differed significantly in SCE counts ($p < 0.001$). Females showed about 1.25 as many SCEs as males. This difference was consistent for nearly all samples and as a result both males and females showed similar temporal and geographic patterns. Differences between the two sexes have been found in other organisms. For instance, in lymphocyte cultures derived from humans exposed to ethylene oxide, cells derived from females had 8% more SCEs than those derived from males (see Evans, 1986).

Again as in earlier studies (Petras and Piscitalli, 1984), no correlation was observed between the SCE levels and the quantity of BrdU absorbed if the amount absorbed fell between 15 and 36mg/mouse. Ninety per cent of the mice examined were in this range.

During the spraying period the increases in SCEs generally ranged from 10% to 50%. The one major exception was the 1985 Trudell west sample; here the SCE level was 90% higher than the control. Evans(1986) comments that in many studies when small samples were exposed to mutagens, the increases of SCEs were often less than a factor of 2. For instance, smokers show between 16 and 30% more SCEs than non-smokers. So our results fall within these levels and suggest that although mutagenic agents are present in the environment the levels are such as to increase SCE levels only slightly. No persistent crisis situation has as yet been encountered.

## POPULATION ENCLOSURES

Previous studies with outdoor population enclosures were short term; animals were placed in these containers for a month or less. These outdoor enclosurers are 90 litre galvanized garbage cans half filled with corn and covered with hardware cloth tops. The corn provides the mice with sufficient water, nutrients and protection. Animals are able to survive even midwinter conditions. These containers were placed at various sites in southwestern Ontario.

In 1984, mice had been placed in enclosures at four different sites: Renaud, Laramie, Rocheleau-McKim and Trudell. There appeared to be no geographic differences in SCE counts in these four regions in that year but there were seasonal differences. The SCE levels were higher in June than in any other period and they appear lowest in November.

In the past year we have begun a longer term experiment. Mice are being left in the enclosures four months instead of one or less. We are attempting to verify the patterns observed in 1985. This experiment was begun by putting mice in the containers August,1985, after the spraying season, and leaving them until mid-December. Three geographically distinct sites were used: Laramie (western Essex County) which gave counts and standard error of the mean of 4.14 and 0.18 per cell; McKim (central part of the county) which gave 2.97±0.36 SCEs per metaphase and Trudell (north-eastern region of the county) 4.28±0.28. The McKim values are the lowest observed in mice living under natural or seminatural conditions. This is not entirely unexpected for a winter sample with no recent herbicide contamination and from a site some distance from industrial population sources. The higher level of SCEs at Laramie's could be explained by the proximity of this site to several major industries. The high SCE values for the Trudell sample are unexpected unless they are affected by emission from the nearby small town of Tilbury. The Trudell farm is 35 miles east of Windsor. The enclosure values at Trudell's in 1985, are consistent with the SCE values of wild mice for that year. Both sets of values are high.

Studies on mice inhabiting population enclosures is continuing. One positive attribute of these animals is that they are inbred, therefore genetically identical and so genetic variance does not contribute to the differences between samples. The differences must then have an environmental basis. Similar differences observed between natural populations could then be primarily attributable to environmental factors.

Incidently, winter samples may be best to establish geographic patterns since there are no confounding factors such as the herbicides.

## RELIABILITY OF SCE ESTIMATES IN WILD MICE

### EFFECT OF STRESS ON SCEs IN MICE

Because animals after being caught are left in traps for several hours and are transported to the laboratory in these traps, the effect of stress on SCE's was considered. Some data on rats suggests that SCEs in these animals were in fact increased by stress.

Three-month old C3H mice were used in this experiment. They were divided into four treatment groups:

a) animals that were shaken hourly for a 15 minute duration on a mechanical shaker beginning four hours after BrdU implantation until the injection of colchicine 20 hours later.

b) animals that were forced to swim for a six minute period every two hours beginning four hours after BrdU implantation and ending with the injection of colchicine.

c) animals that were exposed to shaking for 15 minutes followed the next hour with swimming for 6 minutes. Again this treatment began four hours after BrdU implantation and continued with alternating shaking and swimming hourly until the injection of colchicine.

d) the control group that was composed of mice housed quietly in a cage nearby.

The SCE values in the treated animals did not differ significantly from those in the control group (Table 3), suggesting that exposure to limited stress had no significant effect on SCE levels. The combination of swimming and shaking should perhaps be studied further because it is in this group that the SCE levels were the highest.

The effect of car transportation on SCE levels in captured mice was also considered. On two occasions 3 C3H mice were placed in traps and transported by car to trapping sites and then back to the laboratory. None of the mice showed high SCE values. The mean (4.67 ± 0.87) did not differ significantly from the control group (4.45 ± 0.64).

There is, therefore, no indication that mild physical stress had an effect on SCE levels in inbred mice. As mentioned above the combination of swimming and shaking should be re-examined perhaps for a longer term. Also both of these experiments should be repeated using recently caught wild mice or at least laboratory-born descendents of wild mice. Finally, we should also stress the animals before BrdU implanting.

## AGE AND SCE LEVELS

Mice collected from natural populations obviously vary in age. It is, therefore, essential to determine if there is correlation between SCE levels and age. Reimer and Singh (1983a) studying inbred strains C3H, C57BL/6J and Balb/c found a decline in SCEs induced by cyclophosphamide as the mice increased in age. However, no significant differences were observed in baseline SCEs of 11 and 33 week old mice. Differences due to age have been reported for human fibroblasts (Schneider and Gilman, 1979; Schneider et al, 1979). Age differences have also been seen in the number of micronucleated cells in cultures from donors of different ages. Approximately a four-fold increase has been observed in micronuclei in cultures from an 80-year-old donor when compared to a newborn donor (Fenech and Morley, 1985).

We have examined laboratory-born descendents of wild mice of various ages (Table 4) and found no correlation between age and noninduced SCE levels in mice. The mice were all adults with ages ranging from 2.5 to 9 months. These results are consistent with our earlier findings based on classifying wild mice into two categories: young (less than 15 g) and adult (more than 18 g). The SCE values were 6.28±0.21 for the former and 5.49±0.13 for the latter. This difference was not significant at the 5% level (t = -1.1749).

## VARIANCES OF INDUCED SCE VALUES IN WILD AND INBRED MICE

The induction of SCEs is at least partly under genetic control. Reimer and Singh (1983b) found SCE induction to be controlled by polygenes. Several studies on human populations also indicate genetic control of SCE formation. For instance, there have been reports of differential induction of SCEs by mutagens in normal humans and individuals with xeroderma pigmentosa (Wolff et al, 1977; see also Wolff, 1984). King (1984) gives an overview of genetic susceptibility of humans to chemical exposures.

In natural populations of an organism, unlike the inbred strains, there is considerable genetic variability. The mouse populations of southwestern Ontario are no exception (Petras et al, 1979). This presents two questions about genetic variability. a) Does the level of SCE induction vary from one population to the next and from wild mice to inbred strains? b) Because of the genetic variability of natural populations as compared to inbreds is there a higher variance in SCE values in a sample of wild mice than in inbreds?

To answer both of these queries we compared SCE-induction by different concentrations of cyclophosphamide (CP) in an inbred strain and laboratory-born descendents of wild mice. Table 5 summarizes preliminary results of this experiment. The descendents of wild mice appear to be more susceptable to cyclophosphamide than do C3H mice for all three doses of CP tried. However, the variances of SCE frequencies from one dose to the next and between wild and inbred mice do not show heterogeneity. Bartlet's test of homogeneity of variances shows that the differences observed are not significant at the 5% level.

So although we found a difference in the means of the responses of wild and inbred mice, the variances of each of the groups were similar suggesting that the genetic heterogeneity of wild mice did not contribute to their variance and that the reliability of SCE measurements in wild mice was similar to that of inbreds. The differences in levels of response to SCE-inducing agents and the variability of response will continue to be examined.

## ATTEMPTS TO FIND NATURAL (NON-EXPERIMENTAL) INDUCERS OF SCEs

### THE EFFECT OF HERBICIDES ON SCE IN MICE

Since mice collected during the spraying season gave higher SCE values than those collected before or after, three herbicides widely used in southwestern Ontario were initially selected for a closer examination of their effects.

These were:

Lasso (alachlor; 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide) which is used as a preemergence treatment for corn and soybeans. It controls annual grasses.

Basogran (bentazon; 3-iso-propyl-2,1,3-benzothiadiazin-(4)-one-2, 2-dioxide) which is a postemergence herbicide recommended for control of various broadleaf weeds in peas, flax and in all beans and corn.

Kil-Mor is the trade name of a product composed of three herbicides: 2,4-D (2,4-dichlorophenoxyacetic acid), Mecoprop (d,1-2-(4-chloro-o-tolyloxy) -propionic acid) and Dicamba (3,6-dichloro-o-anisic acid. This mixture is used to control 'hard to kill' broadleaf and grasses in spring wheat, winter wheat, barley, flax, corn and oats.

The mutagenic activities of these herbicides have been evaluated to some extent. All have given negative results in the bacterial reversion-assay with five strains of Salmonella typhimurium and a strain of Escherichia coli (Moriya et al, 1983). Lasso has, however, been found tumorogenic in mice and 2,4-D has significantly increased SCE values in human lymphocytes exposed to 50ug/ml in the medium (Korte and Jalal, 1982). No information is available on possible syngergistic interactions of these agents.

Mice of the inbred strain, C3H, were injected intraperitoneally with either a pesticide dissolved in saline or just saline 12 hours after the implantation of BrdU tablets. The pesticide/saline injections were followed by colchicine injections 12 hours later and the mice were sacrificed 3 hours after the colchicine was given.

Preliminary studies showed that 300mg of Lasso/kg body weight injected intraperitoneally was a lethal dose whereas 150mg/kg was tolerated by the mice. As a result the mice were given 150, 75, 37.5 and 18.75mg/kg body weight. For

Basogran 600mg/kg was found to be lethal whereas 500mg/kg left one of four mice alive and 400 and 300mg/kg did not kill any mice. For Kil-Mor 291mg/kg body weight was toxic whereas 194 and 97mg/kg body weight were not.

The SCE results are given in Table 6. Although in all cases the highest dose of herbicide gave the highest SCE values, the differences between any herbicide-injected animals and saline-injected mice was not significant at the 5% level. If there is a relationship between SCE levels and these herbicides it is, at best, very subtle and would require a much larger sample size to establish. At present we must conclude that these herbicides are not responsible for the SCE shifts seen in natural populations of mice.

There are other herbicides and insecticides being used in the area. Several others Sevin, Chlordane and Diazinon are currently being tested and others will be in the future.

## INDUSTRIAL SETTING AND SCE LEVELS

Three industrial sites were selected to see whether significant increases in SCE levels could be detected. A plastics-molding plant was included because such chemicals as polyvinyl chloride (Anderson et al, 1981), propylene oxide (Hogsted et al, 1983) and ethylene oxide (Hardin et al, 1983) associated with the plastics industry, have been found to be mutagenic and SCE inducers. Also a diesel-repair garage was chosen because the Ames Salmonella Test has revealed that such products of combustion as 9-nitroanthrocene and 1-nitropyrene caused marked induction of revertents (Pitts et al, 1982). Finally, a chrome-plating plant was picked because hexavalent chromium has caused gene mutations (Ohno et al, 1982; Bianch et al, 1983).

In each case six C3H male mice were placed in five gallon, plastic pails containing corn. The corn provided the mice with food, water and shelter. There was sufficient corn to maintain the animals for six weeks. The plastic containers housing the mice were placed in each of the work environments for two weeks. An identical container was kept as a control in the animal facility at the University of Windsor. Bone marrow cells were prepared for SCE analysis in the manner described earlier in this report.

The results are given in Table 7. The SCE counts in mice housed in the plastics-molding plant and the diesel garage showed modest but significant (5% level) deviations from the controls. These differences are not striking and as result the study should be repeated with a larger sample.

As a follow up to the in situ studies some of the more widely used chemicals, that is chemicals with which the workers came in contact in their daily activities were injected into C3H males two hours after the implantation of BrdU tablets. These included:

a) Waste Heat Transfer Oil - a highly stable oil that is used to heat molds used in production. This oil was diluted with an equal volume of corn oil.

b) Coolant - a chemical added to the water used in cutting steel in the process of building molds. This chemical was dissolve in an equal volume of 0.95% saline.

c) Chromic acid - an acid routinely used in a chrome plating plant.

The results are summarized in Table 8. The chromic acid more than doubled the SCE count. This was significant at the 1% level. The transfer oil increased the SCE level at about the 5% level of significance.

The chromic acid results are consistent with data in the literature.

Both the in situ exposure and the injection results suggest that in fact the SCE assay is sufficiently sensitive to uncover genotoxins in the industrial environment. However, the situations that we have uncovered in this study show only subtle increases in SCEs.

## TREATMENT WITH RIVER WATER

In an effort to test the effectiveness of the SCE assay in monitoring genotoxicity of naturally occurring situations, water was obtained in September 1985 from two sources, the Detroit River (foot of the Ambassador Bridge) and Little River ( one mile from its mouth). The Detroit River receives water from the upper Great Lakes including the St. Clair River. Little River is a small stream in the eastern section of Windsor, Ontario. It drains a number of industrial complexes including a chrome-plating plant and a fairly large agricultural area. This river is known as having a serious pollution problem. In the summer months there is frequently a heavy die-off of fish in this stream.

The mice were divided into two treatment groups and a control. Six animals were given five daily intraperitoneal injections of 10 ml/kg of raw water from either the Detroit River or Little River on consective days. The controls were injected with distilled water. The last injection was given 6 hours after the implantation of BrdU tablets. The same procedure was followed in a second run but this time all the water samples were concentrated five-fold by heating.

The results are summarized in Table 9. 'Raw' (unconcentrated) water from both rivers gave higher SCEs than the controls which were given distilled water. These differences are , however, not statistically significant. River waters concentrated by heating gave SCE values lower than that seen in their controls. A comparison of raw water and concentrated water from each source showed a siginificant difference for the Detroit River sample ($t=2.75$; d.f.=10; $p<0.05$). The difference between the two Little River samples was not significant. The difference in the latter case may be an underestimate because the control values for the heated water was 10% higher than for the unconcentrated water. Adjustment of the Little River 'postheating' values to compensate for the high control values would make the difference between the two Little River samples significant at the 6% level (t=2.17; d.f.=9). The difference between the SCEs induced by concentrated Detroit River water and its respective contol is significant (t=2.56; d.f.=10; $p < 0.05$). Unexpectedly these results indicated the Detroit River water to be less genotoxic than concentrated distilled water.

These experiments should be repeated with a larger sample size to determine if heating and concentrating the river waters has a real and persistent effect on the water's genotoxicity.

## ENHANCEMENT OF SENSITIVITY OF SCE ASSAY

### MUTAGEN INHIBITORS

Some preliminary experiments have been carried out with known inhibitors in an attempt to reduce the baseline count. If a reduction were seen this would be an indication that the mice in our animal facilities are exposed to genotoxic agents.

The inhibitors used were selenium (5.43mg/l) and ascorbic acid (25g/l). Both were dissolved together in drinking water and combined with sucrose (20/l). Sucrose was added to make the water more palatable. The mice were placed on this solution 16 days before BrdU was implanted. Six hours after the implantation cyclophosphamide (CP) (10mg/l) or saline were injected intraperitoneally.

No decrease in SCEs was observed in the baseline count (see Table 10) suggesting that either no inducing agent was acting on the baseline count or that the inducer(s) were not affected by the inhibitor. The inhibiting solution did affect the CP-induced SCE levels. The decrease was significant at the 1% level. Which of the two compound is responsible for the reduction remains to be determined.

## MUTAGEN PROMOTORS

In an attempt to increase the sensitivity of the SCE assay we have also begun to examine some common mutation promotors.

Based on a survey of the literature we began to look at three compounds which have been commonly used in the past. These were arsenic (8mg/kg/injection), caffeine (80mg/kg/injection) and cyclamic acid (300mg/kg/injection). Each C3H mouse was given three injections at 8, 11, and 14 hours after BrdU implantation. The mutagen, if given, was given 7.5 hours after implantation.

Alone none of the three compounds increased the SCE count significantly over the baseline level (see Table 11). Cyclamic acid and arsenic appeared to inhibit CP induction of SCEs and caffeine appeared to have no effect. For mitomycin C , caffeine appeared to increase SCE induction slightly. The other two had no effect. Based on these results, we considered none of these compounds to be good candidates for increasing the sensitivity of the SCE assay.

## A SECOND MONITORING SYSTEM

Several individuals have suggested the need for a second monitoring system to confirm the results of the SCE assays. Of the systems available we have examined the MICRONUCLEUS (MN) ASSAY FOR PERIPHERAL BLOOD. This assay has been described as less sensitive than the SCE assay by some (Bauknecht et al, 1977; Lasne et al, 1984) and equally sensitive by others (Cole et al, 1983). Furthermore it may be considered complementary to the SCE assay because SCE and MN inductions appear to be independent (Renault et al, 1982). Finally, the MN assay of peripheral blood is the backup procedure of choice because of properties (see below) which should prove valuable in studies of natural populations, in continuous monitoring of long term exposure effects and in genetic analyses. The selection of the MN assay was further enhanced by the acridine orange fluorescent staining technique describe by Hyashi et al (1983) and Tice (1985). This procedure appears to eliminate many of the scoring problems associated with Giemsa. As a result, we have begun to develop a time-response profile for several genotoxins to determine optimal bleeding times and we have also begun to determine the frequencies of micronucleated erythrocytes of wild mice and treated inbred animals for comparison with SCE data.

## MICRONUCLEUS ASSAY-CHARACTERISTICS

Heddle (1973) has described the micronucleus assay as simpler, faster and easier to score than other cytological monitors of genotoxicity (see also: Salamone and Heddle, 1983). Generally micronuclei are formed as fragments of chromosomes which have lost their centrioles and therefore lag behind in the cytoplasm when the nucleus is reformed. A second, perhaps less common process, involves the detachment of a whole chromosome from the spindle apparatus. This second mechanism may be increased through the use of spindle poisons (Schmid, 1975).

Usually micronuclei have been assayed in polychromatic cells (PCEs) of bone marrow. These cells occur as the penultimate stage in erythrocyte formation. Tice and Ivett (1985) have summarized the positive features of the bone marrow micronucleus assay. The advantages include: a) micronuclei can be identified quickly in anucleated polychromatic erythrocytes, b) metaphase preparations are not required since interphase cells are used, c) newly formed cells reside in bone marrow only about 24 hours, therefore, any increase in micronucleated polychromatic cells is an indication of a recent exposure to a mutagen and d) there is a strong correlation between cancer-induction and micronucleus induction. The drawbacks of the bone marrow assay include DNA-nonrelated causes of micronuclei and the inability to do sequential sampling since animals are killed to assay bone marrow material.

To overcome the last problem MacGregor et al (1980) suggested assaying for micronuclei in polychromatic (PCEs) and normochromatic erythrocytes (NCEs) of peripheral blood since sacrificing of mice is not required. Furthermore, since PCEs are converted, within 72 hours after their formation in bone marrow, to NCEs, the time when exposure occurred can be divided into two periods, a recent 72 hours period and an earlier 35-50 day period since PCEs have a life span of up to 48 hours in peripheral blood and NCEs have a life span of 35-50 days. Unlike the situation in rats and humans, the cells with micronuclei are not preferentially removed from peripheral blood of mice (MacGregor et al, 1980; Schlegal and MacGregor, 1982). This permits distinction between a single recent exposure to a mutagenic agent and chronic exposure.

## GENERAL PROCEDURE

The basic procedure used was obtained from R.R. Tice (personal communication). The following steps are involved: a) peripheral blood is obtained from a mouse by inserting a heparinized tube into the suborbital sinus; b) a drop of blood is spread evenly on the microscope slide; c) the slide is air dried and fixed in absolute methanol for 15 minutes; d) after 24 hours of aging the slide is stained with acridine orange (0.06125mg/ml of pH 7.4 phosphate buffer) for 3 to 4 minutes, then rinsed in buffer and washed in buffer for 6 minutes; e) the spread is covered carefully, to avoid air bubbles, with a cover slip; f) the slide is then examined under fluorescence microscopy (FITC filter combination, excitation wavelength 495nm, emission wavelength 520nm) and scored for i) number of micronucleated NCEs in 1000 NCEs, ii) number of micronucleated

PCEs in 1000 PCEs, and iii) percentage of PCEs in 1000 erythrocytes.

Under fluorescence, micronuclei which have been stained with acridine orange appear green, NCEs brownish-green and PCEs reddish-orange. If either the fluorescence or background stains are not suitable, the slide can be destained with absolute methanol and restained.

Acridine orange [494-38-2] discriminates between DNA which fluoresces green and RNA which fluoresces red. Acridine orange also binds to acid polysaccharides resulting in bright red fluorescence. These properties make the scoring of micronuclei relatively straightforward (Hayashi et al, 1983).

## TIME RESPONSE STUDY FOR OPTIMAL RESULTS

After obtaining consistently good slide preparations so that scoring micronucleated PCEs and NCEs presented no difficulties, a profile of time-dependent induction of micronuclei was developed for two compounds, Cyclophosphamide (CP) and Mitomycin C (MMC). Other compounds, including methylmethanesulfonate, benzene, 6-mercaptopurine, and 7,12 dimethylbenzantracene, are either being or will be examined. These compounds have been chosen because they have been found to show different time responses in inducing bone marrow micronucleated PCEs (Hayashi et al, 1984; Salamone et al, 1980).

CP (50mg/kg), MMC (2mg/kg) or saline were injected once,intraperitoneally, into four, five month old C3H male mice. Blood samples were taken from the suborbital sinuses. The first samples were taken just before injection and then at regular intervals (see Figure 1). The last blood samples were taken 23 days after the first to see if the micronucleated cells returned to the starting level.

For both CP and MMC the maximum effect on PCEs was reached around the 48 hour period (Figure 1). This is about 24 hours later than in bone marrow cells for the MMC treated mice (Hayashi, 1984) and 12 hours later for CP treated animals (MacGregor et al, 1980). The increase of micronucleated cells was detected in the NCEs between 24 and 48 hours later. This increase of micronuclei in NCEs was not as dramatic as in the PCEs because of the dilution of micronucleated cells. Since NCEs last 35-50 days, the effect of a single injection is diluted about ten-fold. Calculations based on the micronucleated PCEs observed would suggest that from a single injection, at the concentrations used, the maximum micronucleated NCE count should be about 3 (Hayashi et al, 1984).

Another set of data collected was the percent of PCEs observed in the various samples. In the controls the percent of PCEs increased with each bleeding except for the last (552 hrs). This is not unexpected since the production of erythrocytes was stimulated by the removal of blood. In mice injected with CP the same pattern was observed but the production of new PCEs was reduced by almost 50%. MMC appeared to inhibit almost completely the production of new PCEs. By the 23rd day the PCE levels were almost back to the pretreatment values.

This phase of the study suggests that mice should be examined about 48 hours after their exposure to a mutagen and that in the first 24 hours although there is an effect on the frequency of micronucleated PCEs that effect is not nearly as strong as the 48 hour effect.

Wild mice should be examined for micronuclei in peripheral blood PCEs within 48 hours of being trapped. If the exposure to a genotoxic agent was short-term, that is a single pulse, then the micronucleated NCEs should be low, with a peak being reached 24 to 48 hours after the PCE maximum. If the exposure is chronic then the frequency of micronucleated NCEs should be much higher and should persist at a higher level (see Schlegal and MacGregor, 1982; Salamone and Heddle, 1983).

The percent of PCEs in peripheral blood may also be an indicator of agents in the environment which inhibit cell replication in the bone marrow. Just how meaningful this parameter may be, has yet to be determined.

## MICRONUCLEI AND NATURAL POPULATIONS

Table 12 summarizes the MN values observed in the wild mice collected in 1986. Very little variability was observed. No geographic or temporal patterns are evident. This suggests that the MN assay may be less sensitive than the SCE analyses.

## MALATHION, BENZENE AND MICRONUCLEI

Preliminary micronucleus-induction information has been obtained for several other compounds including malathion and benzene. Malathion was examined because it is commonly used to control ectoparasites in laboratory animals and we were concerned about the effect that occasional dunking of mice had on the frequency of micronucleated cells. Malathion has been described as genotoxic when injected. Our experiment involved topical exposure by dunking the mice in a 5% solution of commercially available (Purina) malathion. Malathion did not appear to increase the frequency of micronucleated cells (Table 13).

Benzene was examined as a prelude to developing a 'crisis situation' for SCE studies. Mice were exposed to benzene vapor by placing 0.25ml in the bedding hourly for 6 hours. The mice were examined for micronucleated PCEs and NCEs 48 hours after the last 'treatment'. A 3-fold increase was seen in the micronucleated PCEs (Table 13). These results are consistent with the findings of others.

## EFFECT OF STRESS ON MICRONUCLEI

The same mice used in the SCE assay for the effect of stress (see above) were bled and also assayed for micronuclei. The results summarized in Table 14 appear consistent with the SCE data. The deviations from the control values were not significant.

## INDUSTRIAL SETTINGS AND MICRONUCLEI

The mice that had been placed in the chrome plating plant were also assayed for micronucleated NCEs and PCEs in peripheral blood. See Table 15. These are consistent with the results obtained for the SCE assay (Table 7).

## OCTACHLOROSTYRENE AND RIVER WATER

Octachlorostyrene (OCS) is a byproduct of industrial processes involving carbon and chlorine reactions at high temperature which has found its way into the Great Lakes and has been isolated from fishes (Kuehl et al, 1976) and from herons (Reichel et al, 1977). Both in short-term and long-term studies Chu et al (1984,1986) described this compound as having a serious effect on hepatic microsomal enzymes, serum components, the kidney and the thyroid gland at, in some cases, concentrations as low as 5ppm in rats. Also accumulation of OCS has been found in adipose tissue. No information is available on the genotoxicity of this compound, although Chu et al (1986), in their long-term study, report no induction of tumors attributable to OCS.

Since this compound is found in the Detroit River and was available to us in highly purified form we decided to see what effect it had on micronucleus induction. In our study we injected intraperitoneally into C3H male mice 200, 400 and 800mg/kg of OCS (obtained through Dr. P. Hebert from the Great Lakes

Institute, University of Windsor). The mice were bled at 24 hour intervals beginning just prior to the injection of OCS. The results of the 800mg/kg dose are presented in Table 16. Since the frequency of micronucleated cells at various times after the injection of this dose of OCS does not differ from the frequency prior to the injection, no attempt was made to score animals receiving lower doses.

These findings suggest that octachlorostyrene is not an inducer of micronuclei in mice and therefore probably not mutagenic.

## SUMMARY

1] The SISTER CHROMATID EXCHANGE (SCE) ASSAY is now being used routinely in monitoring wild mice and mice exposed to various treatments or conditions.

2] Monitoring natural populations of the house mouse is continuing. A data base on SCE levels in popultions of various parts of southwestern Ontario is being developed. Both geographic and seasonal patterns have been observed over a four-period. Similar patterns are also being observed in inbred mice placed in population enclosures.

3] The reliability of the SCE values of wild mice to monitor the environment is being examined by studying the effects of stress, age and genetic variability on SCE. All of these indicate that the SCE assay on wild mice is a suitable test for monitoring environmental genotoxicity levels.

4] Various situations have been and are being examined to further test the suitability of the SCE in mice for monitoring the environment for genotoxin levels. These include herbicide-spraying practices, industrial settings and aquatic environments. Increases in SCEs have been seen in a number of cases but even when significant the increases have been modest.

5] In an effort to increase the sensitivity of the SCE assay we have begun to examine known inhibitors and promotors of mutagens/carcinogens for their effects. Of the battery of chemicals available which are known to promote genotoxic activity, the few examined so far appear to have only subtle effects.

6] As a backup or complementary system the MICRONUCLEUS ASSAY OF PERIPHERAL BLOOD appears to be a good candidate. This test appears to be quite sensitive for detecting certain genotoxic agents and has the advantage of being applied serially since the animals are not sacrificed. We are now applying this assay to most of the mice being tested for SCEs.

7] A time-response study has been done on two compounds, cyclophosphamide and mitomycin C, using the Peripheral Blood Micronucleus Assay. Peak effects in terms of the frequency of micronucleated polychromatic erythrocytes (PCEs) are reached 12 to 24 hours after similar peaks are observed in bone marrow tissue.

8] The Peripheral Blood Micronucleus Assay has also been applied to several experimental situations, in some cases as a followup to the SCE work while in others, preceding SCE studies. Malathion, octachlorostyrene and some of the industrial exposures had no effect on the frequency of micronucleated PCEs while benzene did.

## CONCLUSIONS

The work is progressing well in a number of different areas. The SISTER CHROMATID EXCHANGE ASSAY is now pratical for samples of 30 animals and gives consistent results. The PERIPHERAL BLOOD MICRONUCLEUS ASSAY with acridine orange as the stain should serve as an excellent complement to the SCE test.

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Figure 1: The effect of cyclophosphamide and mitomycin C on micronucleated a) polychromatic erythrocytes (PCE), b) normochromatic erythrocytes (NCE) in peripheral blood, and c) the production of new PCEs.

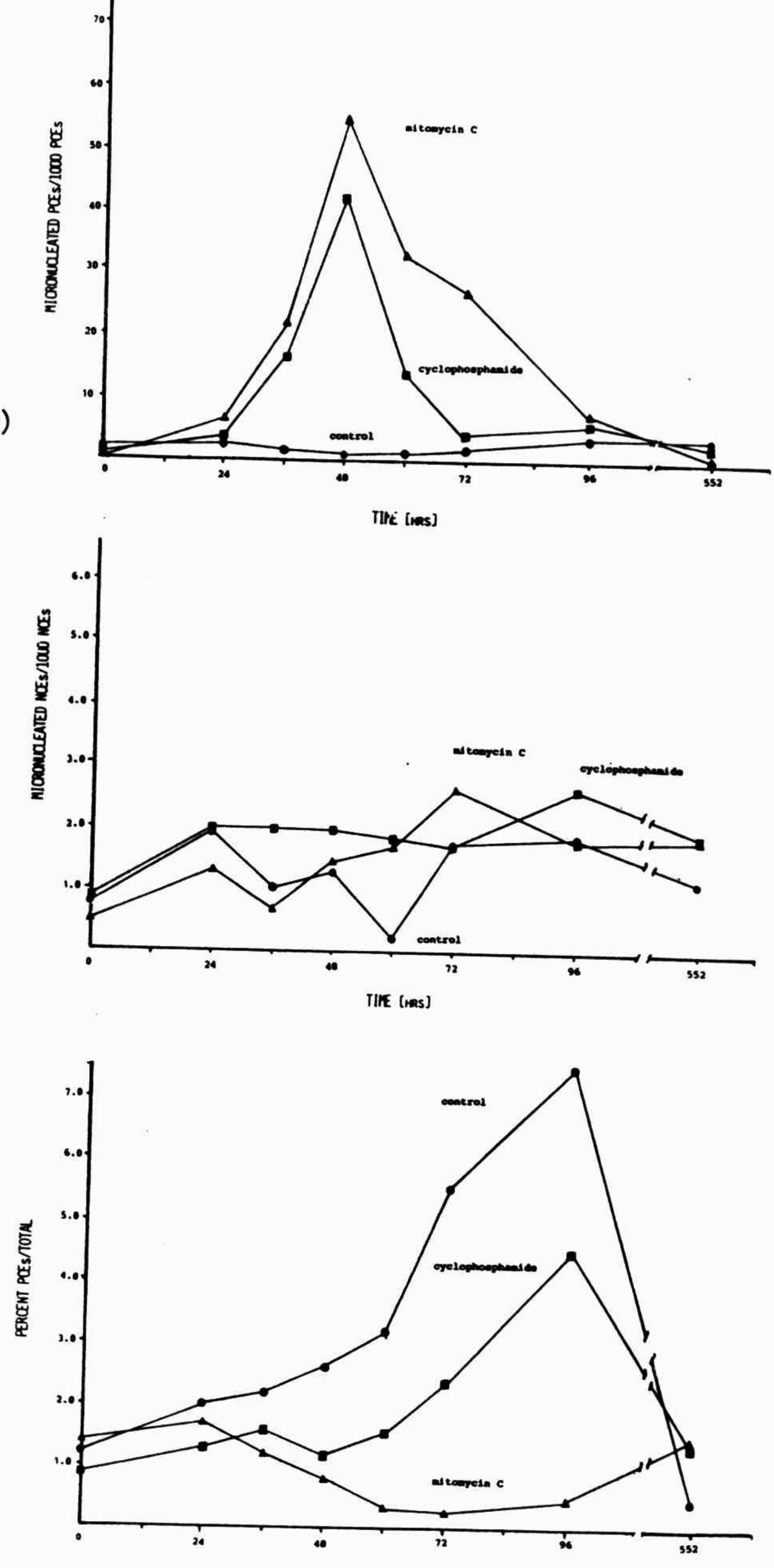

TABLE 1a. SCE levels in wild mice collected from corn cribs in three regions of southwestern Ontario before, during and after the spraying of crops with herbicides during 1986. Since there was a significant differences between the sexes, the sexes are considered separately.

| | | SCE COUNTS + S.D. | | | | | |
|---|---|---|---|---|---|---|---|
| LOCALITY | DATE | PRESPRAYING | | SPRAYING | | POST-SPRAYING | |
| | | FEMALES | MALES | FEMALES | MALES | FEMALES | MALES |
| ESSEX COUNTY: (86) | | | | | | | |
| McKim | 4/18 | 5.35(6)<br>±1.06 | 4.18(4)<br>±0.50 | | | | |
| Renaud | 5/26 | 4.84(5)<br>±0.50 | 4.15(5)<br>±0.38 | | | | |
| Amlin | 6/04 | | | 6.72(10)<br>±1.39 | 6.24(6)<br>±0.97 | | |
| Goulin | 6/10 | | | 8.46(6)<br>±1.00 | 6.69(6)<br>±1.02 | | |
| Holden | 6/11 | | | 7.18(3)<br>±0.75 | 5.38(3)<br>±0.66 | | |
| Farrough | 8/04 | | | | | 5.75(10)<br>±1.41 | 5.81(9)<br>±0.69 |
| Boide | 9/04 | | | | | 7.00(7)<br>±1.57 | 5.70(6)<br>±0.61 |
| Total | | 5.12<br>±0.86 | 4.16<br>±0.41 | 7.34<br>±1.40 | 6.19<br>±1.01 | 6.26<br>±1.23 | 5.76<br>±0.52 |
| KENT COUNTY: (44) | | | | | | | |
| Dilliot | 5/08 | 5.98(2)<br>±0.11 | 5.28(2)<br>±0.46 | | | | |
| Lucio | 5/21 | 6.20(5)<br>±1.36 | 5.21(5)<br>±1.13 | | | | |
| Sheldon | 5/14 | | 5.22(2)<br>±0.32 | | | | |
| Oskar | 6/23 | | | 5.30(7)<br>±0.78 | 4.85(4)<br>±0.47 | | |
| Cl'dening W | 7/08 | | | 6.63(2)<br>±1.17 | 5.67(7)<br>±1.41 | | |
| Bokor | 7/08 | | | 7.43(4)<br>±1.39 | 6.35(4)<br>±1.66 | | |
| Total | | 6.14<br>±1.11 | 5.23<br>±0.82 | 6.09<br>±1.35 | 5.63<br>±1.34 | | |

Number in parenthesis is the size of the sample.

TABLE 1b. SCE levels in wild mice collected from corn cribs in three regions of southwestern Ontario before, during and after the spraying of crops with herbicides during 1985. Since there was a significant differences between the sexes, the sexes are considered separately.

| LOCALITY | DATE | SCE COUNTS + S.D. | | | | | |
|---|---|---|---|---|---|---|---|
| | | PRESPRAYING | | SPRAYING | | POST-SPRAYING | |
| | | FEMALES | MALES | FEMALES | MALES | FEMALES | MALES |
| **ESSEX COUNTY:** | | | | | | | |
| McKim | 5/11 | 5.48(4)<br>±0.46 | 5.41(4)<br>±0.21 | | | | |
| Borrowman | 5/20 | 6.30(3)<br>±0.42 | 4.71(4)<br>±0.73 | | | | |
| Rocheleau | 5/16 | | 4.53(5)<br>±0.33 | | | | |
| Goolan | 5/23 | 5.21(6)<br>±0.36 | 4.65(4)<br>±0.27 | | | | |
| Renaud | 6/8 | | | 5.52(2)<br>±0.03 | 5.45(2)<br>±0.60 | | |
| Wright | 6/8 | | | 6.52(3)<br>±0.78 | 6.05(1) | | |
| Trudell | 5/31 | | | 9.03(4)<br>±1.81 | 6.53(4)<br>±0.65 | | |
| Trudell E. | 7/22 | | | 9.22(3)<br>±1.06 | 4.99(4)<br>±1.52 | | |
| Pook | 7/31 | | | | | 11.00(2)<br>±2.50 | |
| Boide | 8/27 | | | | | 7.40(3)<br>±0.83 | 5.23(5)<br>±0.60 |
| Total | | 5.54(13)<br>±0.25 | 4.93(17)<br>±0.33 | 7.92(12)<br>±0.74 | 5.70(11)<br>±0.59 | 8.84(5)<br>±1.24 | 5.58(5)<br>±0.53 |
| **KENT COUNTY:** | | | | | | | |
| Allen | 6/11 | | | 6.13(7)<br>±0.54 | 5.55(5)<br>±0.47 | | |
| McKinley | 6/17 | | | 7.20(2)<br>±0.15 | 5.95(4)<br>±0.36 | | |
| Oskar | 6/17 | | | 6.85(3)<br>±0.43 | 5.92(5)<br>±0.18 | | |
| Clenndening | 6/18 | | | 7.00(2)<br>±0.60 | 5.23(4)<br>±0.45 | | |
| Cl'dening W | 6/26 | | | 5.90(2)<br>±0.05 | 4.53(3)<br>±0.20 | | |
| Total | | | | 6.34(16)<br>±0.25 | 5.51(21)<br>±0.18 | | |
| **ELGIN COUNTY:** | | | | | | | |
| Brown | 7/3 | | | 4.81(4)<br>±0.47 | 4.68(3)<br>±0.48 | | |

Number in parenthesis is the size of the sample.

TABLE 2. SCE levels in wild mice collected from corn cribs in three regions of southwestern Ontario before, during and after the spraying of crops with herbicides over a four year period. Mice collected in 1984, 1985 and 1986 had 50 mg BrdU tablets implanted for SCE staining whereas the 1983 were injected with 80 mg of BrdU.

| | | SCE COUNTS + S.E.M. | | |
|---|---|---|---|---|
| YEAAR | LOCALITY | PRESPRAYING | SPRAYING | POST-SPRAYING |
| 1986 | Essex County | 4.64±0.22 (20) | 6.76±0.16 (34) | 6.01±0.24 (32) |
| | Kent County | 5.69±0.41 (27) | 5.86±0.38 (17) | |
| 1985 | Essex County | 5.16±0.16 (30) | 7.07±0.43 (24) | 7.21±0.84 (10) |
| | Kent County | | 5.92±0.36 (37) | |
| | Elgin County | | 4.74±0.31 (7) | |
| 1984 | Essex County | 5.70±0.19 (28) | 6.35±0.17 (32) | |
| | Kent County | 4.65±0.43 (19) | 6.66±0.24 (38) | 4.89±0.18 (30) |
| | Elgin County | | 6.62±0.46 (20) | |
| 1983 | Pooled data | 5.99±0.32 (12) | 9.22±0.86 (10) | |

The numbers in parentheses are the sample sizes.

Table 3. Effect of Stress on Sister Chromatid Exchange in C3H males

| Treatment | Number of Mice | SCE ± SEM |
|---|---|---|
| Control | 4 | 4.28 ± 0.30 |
| Swimming only | 3 | 4.78 ± 0.54 |
| Shaking only | 3 | 4.40 ± 0.28 |
| Swimming and Shaking | 3 | 5.55 ± 0.49 |

Table 4 Correlation between age and SCE levels in laboratory-born descendents of wild mice.

| | Laboratory born descendents of Wild mice | | |
|---|---|---|---|
| Source | Age | Number of Mice | SCE Values ± SEM |
| Houle | 2 1/2 months | 6 | 6.2099 ± 0.54 |
| Borrowman E. | 3 months | 3 | 6.6333 ± 0.62 |
| Houle | 4 months | 6 | 6.4083 ± 0.49 |
| Houle | 5 months | 6 | 4.8021 ± 0.44 |
| Paquette | 7 months | 6 | 5.0250 ± 0.17 |
| Paquette | 9 months | 6 | 6.1167 ± 0.54 |

There is no significant correlation between age and SCE values.

**Table 5.** Variances of SCE values in wild and C3H mice given varying amounts of cyclosphosphamide.

| Mice | CP Level | Number of Mice | SCE | Variance |
|---|---|---|---|---|
| Wild Offspring | 0.00 | 4 | 6.1625 | 1.9293 |
| | 0.045 | 11 | 5.350 | 0.9149 |
| | 0.45 | 4 | 7.8750 | 3.5608 |
| | 4.5 | 9 | 18.0777 | 4.7961 |
| C3H | 0.00 | 4 | 4.1825 | 1.4938 |
| | 0.045 | 4 | 4.3375 | 0.4255 |
| | 0.45 | 5 | 6.2500 | 0.4662 |
| | 4.5 | 4 | 12.6125 | 6.5485 |

Bartlet's test of homogeneity of variances shows that the differences are not significant.

TABLE 6: Mean SCE values in C3HeB/FeJ male mice receiving intraperitoneal injections of herbicides dissolved in saline or just saline.

| TREATMENT (mg/kg body weight) | | NO. OF MICE | MEAN SCE/CELL ± SEM | |
|---|---|---|---|---|
| Saline | | 15 | 4.96 | 0.33 |
| Lasso | 150 | 4 | 6.01 | 0.51 |
| | 75 | 4 | 4.23 | 0.12 |
| | 37.5 | 4 | 4.58 | 0.17 |
| | 18.75 | 4 | 5.31 | 0.54 |
| Basogran | 500 | 1# | 5.90 | |
| | 400 | 4 | 4.51 | 0.25 |
| | 300 | 4 | 5.06 | 0.52 |
| Kil-Mor | 194 | 4 | 5.40 | 0.21 |
| | 97 | 4 | 4.32 | 0.34 |

# Only one of four survived the treatment.

TABLE 7: Mean SCE values in C3H male mice placed in cages partially filled with corn in three different industrial settings.

| SITE | NO. OF MICE | MEAN SCE/CELL ± SEM | |
|---|---|---|---|
| University (controls) | 6 | 4.61 | 0.04 |
| Plastics-molding plant | 5 | 5.63 | 0.27* |
| Diesel garage | 6 | 7.05 | 0.53* |
| Chrome plating plant | 7 | 5.47 | 0.02 |

* Deviation from controls significant at the 5% level.

TABLE 8: Mean SCE values in C3H male mice injected with three common chemical found in the industrial settings studied.

| CHEMICAL | NO. OF MICE | DOSE | MEAN SCE/CELL ± SEM | |
|---|---|---|---|---|
| Transfer oil | 3 | 3.75 ml/kg | 6.05 | 1.15* |
| Coolant | 3 | 3.75 ml/kg | 5.67 | 1.48 |
| Chromic acid | 3 | 15 mg/kg | 9.05 | 0.80** |
| Control | 3 | | 4.43 | 0.19 |

* Deviation from controls significant at the 5% level.
** Deviation from controls significant at the 1% level.

TABLE 9: Mean SCE values in C3H male mice receiving five daily injections of water obtained from the Detroit River and Little River, a small stream that drains several industrial complexes including a chrome plating factory. The water from the rivers and the distilled water injected into the controls was either raw (unconcentrated) or concentrated five-fold by heating.

| TREATMENT | NO. OF MICE | MEAN SCE/CELL ± SEM | |
|---|---|---|---|
| UNCONCENTRATED WATER (raw): | | | |
| Controls | 5 | 5.12 | 0.23 |
| Detroit River | 6 | 6.77 | 0.69 |
| Little River | 6 | 6.47 | 0.55 |
| CONCENTRATED WATER (heated): | | | |
| Controls | 5 | 5.72 | 0.42 |
| Detroit River | 6 | 4.51 | 0.18 |
| Little River | 5 | 5.19 | 0.31 |

Table 10. Effect of several mutagen inhibitors on the SCE levels in C3H mice.

| Treatment | Number of Mice | SCE ± SEM |
|---|---|---|
| Control (No inhibitor No Cyclosphosphamide) | 4 | 4.36 ± 0.39 |
| Cyclosphosphamide only | 4 | 30.51 ± 0.35 |
| Inhibitor only | 4 | 4.90 ± 0.41 |
| Cyclosphosphamide and Inhibitor | 4 | 25.89 ± 0.10 |

Table 11. Effects of mutagen promoters on SCE levels induced by CP and MMC.

| Promoter | Mutagen | Number of Mice | SCE ± SD |
|---|---|---|---|
| Saline | Saline | 6 | 4.81 ± 0.15 |
| Caffeine | Saline | 2* | 4.97 ± 0.42 |
| Cyclamic Acid | Saline | 3 | 5.25 ± 0.25 |
| Arsenic | Saline | 3 | 4.88 ± 0.25 |
| Saline | Cyclosphosphamide | 3 | 15.17 ± 0.76 |
| Caffeine | Cyclosphosphamide | 3 | 14.80 ± 0.80 |
| Cyclamic Acid | Cyclosphosphamide | 3 | 12.58 ± 0.76 |
| Arsenic | Cyclophosphamide | 3 | 13.33 ± 0.89 |
| Saline | Mitomycin C | 3 | 17.03 ± 0.43 |
| Caffeine | Mitomycin C | 3 | 21.02 ± 1.60 |
| Cyclamic Acid | Mitomycin C | 3 | 15.20 ± 1.29 |
| Arsenic | Mitomycin C | 2 | 16.70 ± 0.95 |

* Where less than 3 mice were scored for SCEs deaths had occurred.

TABLE 12. Results of micronucleus analyses in polychromatic and normochromatic erythrocytes of peripheral blood obtained from the suborbital sinus of mice caught in 1986. The last column is a measure of the formation of new erythrocytes.

| LOCATION | NO. | DATE OF CAPTURE | MEAN MN-PCE PER 1000PCE | MEAN MN-NCE PER 1000NCE | MEAN PERCENT PCE PER TOTAL |
|---|---|---|---|---|---|
| **Essex County** | | | | | |
| **McKim east** | 18 | 4/18/86 | 1.004±1.174* | 0.728±0.77 | 2.714±1.871 |
| **McKim west** | 17 | 4/25/86 | 1.685±1.376 | 0.767±0.553 | 4.077±2.328 |
| **Renaud north** | 20 | 5/28/86 | 0.628±0.784 | 0.948±0.778 | 2.708±3.523 |
| **Renaud middle** | 13 | 5/28/86 | 2.189±2.404 | 0.272±0.665 | 2.142±1.523 |
| **Amlin** | 16 | 6/6/86 | 1.894±1.401 | 0.903±0.766 | 1.686±1.083 |
| **Goolan north** | 5 | 6/12/86 | 0.730±0.732 | 0.536±0.787 | 0.536±0.439 |
| **Goolan south** | 7 | 6/12/86 | 0.954±1.114 | 0.933±0.794 | 1.064±0.637 |
| **Holden** | 8 | 6/13/86 | 1.479±1.057 | 1.090±0.580 | 1.718±0.795 |
| **C. Wilkinson** | 9 | 6/26/86 | 1.378±0.500 | 1.570±1.153 | 1.912±1.046 |
| **Kent County** | | | | | |
| **Dilliot** | 4 | 5/9/86 | 0.680±0.844 | 1.133±1.229 | 2.168±2.064 |
| **Lucio** | 28 | 5/23/86 | 2.720±1.220 | 0.566±0.814 | 3.669±2.518 |
| **Sheldon** | 5 | 5/23/86 | 1.532±1.494 | 1.648±2.956 | 1.504±1.081 |
| **Whittington** | 3 | 6/9/86 | 0.903±0.926 | 0.947±1.125 | 2.587±1.761 |
| **Elgin County** | | | | | |
| **Oskar** | 16 | 6/20/86 | 0.770±0.758 | 1.730±1.552 | 3.629±5.829 |
| **Virog** | 1 | 6/26/86 | 3.920 | 1.450 | 1.010 |
| **Controls** | | | | | |
| **C3HeB/FeJ** | 3 | 5/9/86 | 2.333±0.586 | 1.630±0.26 | 1.593±0.659 |
| | 4 | 5/22/86 | 1.554±2.055 | 1.117±0.925 | 1.830±0.348 |
| | 3 | 5/28/86 | 2.103±1.489 | 1.393±1.660 | 2.683±2.130 |
| | 4 | 6/6/86 | 2.790±2.500 | 1.013±0.900 | 1.513±0.304 |
| | 4 | 6/12/86 | 2.432±2.094 | 0.852±0.102 | 1.215±0.317 |
| | 4 | 6/25/86 | 1.830±0.794 | 0.845±0.670 | 2.385±0.447 |
| | 4 | 6/26/86 | 0.688±0.863 | 1.135±0.591 | 1.795±0.464 |

* Standard deviation.

TABLE 13: Induction of micronuclei per 1000 cells in peripheral blood of male C3H mice by immersing the mice in a 5% malathion solution or exposing them to benzene vapours.

| TREATMENT | NO. OF MICE | MICRONUCLEATED NCEs | MICRONUCLEATED PCEs | PERCENT PCEs |
|---|---|---|---|---|
| Controls | 4 | 0.78<br>±1.06* | 1.77<br>±0.71 | 1.20<br>±0.63 |
| Malathion | 4 | 0.47<br>±0.66 | 1.34<br>±1.89 | 3.50<br>±3.40 |
| Benzene | 4 | 1.72<br>±1.35 | 5.79<br>±0.64 | 1.69<br>±0.95 |

* Standard deviation.

TABLE 14: Micronuclei per 1000 cells in peripheral blood of C3H male mice exposed to 15 minutes of shaking per hour for 16 hours, 6 minutes of swimming every two hours for 16 hours or alternating the shaking and swimming hourly. The stress was begun 4 hours after the implanting of the BrdU tablet and continue until the injection of colchine.

| TREATMENT | NO. OF MICE | MICRONUCLEATED NCEs | MICRONUCLEATED PCEs | PERCENT PCEs |
|---|---|---|---|---|
| Controls | 4 | 1.22<br>±0.52* | 2.00<br>±0.86 | 1.61<br>±0.33 |
| Shaker only | 3 | 2.18<br>±0.69 | 2.80<br>±1.03 | 1.26<br>±0.59 |
| Shaker & Swimming | 3 | 1.15<br>±0.57 | 0.81<br>±1.14 | 2.70<br>±2.79 |
| Swimming only | 3 | 1.72<br>±1.06 | 1.11<br>±1.34 | 4.88<br>±4.89 |

* Standard deviation.

TABLE 15: Micronuclei per 1000 cells in peripheral blood of C3H male mice housed in large plastic cages partly filled with corn. Five mice were placed in eachcage. These containers were place in a chrome plating plant within 3 meters of the plating vats. The control mice were housed in a similar container in the animal facility at the University.

| TREATMENT | NO. OF MICE | MICRONUCLEATED NCEs | MICRONUCLEATED PCEs | PERCENT PCEs |
|---|---|---|---|---|
| Controls | 4 | 0.23<br>±0.45* | 2.28**<br>±2.31 | 1.44<br>±0.34 |
| Chrome plating plant | 10 | 1.69<br>±1.16 | 1.95<br>±1.06 | 2.52<br>±0.56 |

* Standard deviation.

** This high value was due primarily to one mouse which had 5.66 micronucleated PCEs/1000. Without this mouse the count would have been 1.20 ± 0.56.

TABLE 16: Micronuclei per 1000 cells in peripheral blood of C3H male mice receiving 800 mg/kg octachlorostyrene.

| TIME AFTER INJECTION | NO. OF MICE | MICRONUCLEATED NCEs | MICRONUCLEATED PCEs | PERCENT PCEs |
|---|---|---|---|---|
| 0 hours | 4 | 2.55<br>±0.94* | 0.98<br>±0.77 | 2.27<br>±1.03 |
| 48 hours | 4 | 1.86<br>±1.04 | 1.43<br>±1.18 | 3.77<br>±3.20 |
| 72 hours | 4 | 1.43<br>±1.85 | 1.15<br>±0.98 | 2.50<br>±0.94 |
| 96 hours | 4 | 2.41<br>±1.12 | 1.93<br>±0.99 | 3.19<br>±1.59 |
| 120 hours | 4 | 1.12<br>±0.50 | 0.87<br>±1.39 | 3.08<br>±1.08 |
| 144 hours | 4 | 0.90<br>±0.75 | 0.60<br>±0.47 | 3.45<br>±1.23 |

* Standard deviation.

# Field and Laboratory Validation of a HiVol Denuder for Minimizing PAH-Oxidant Reactions During HiVol Sampling

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## INTRODUCTION

Results obtained in a recent study "Evaluation of Alternatives to HiVol Sampling for Polynuclear Aromatic Hydrocarbons", by Concord Scientific Corporation for the Ontario Ministry of the Environment, Air Resources Branch, showed that a denuder efficiently removes ozone from an air stream prior to a HiVol filter (CSC, 1984). In a single experiment, the ozone denuder appeared to reduce on-filter reactions between ozone and captured PAH, as indicated by the significant drop in the number of oxy-PAH species on a protected filter compared to an unprotected filter.

### Study Objectives

The objective of this study was to evaluate further the HiVol ozone denuder in terms of mass and the relative attenuation of PAH and PAH derivatives, the effectiveness for removal of $NO_2$ and $HNO_3$, and the identification of the oxy- and nitro-PAH contained in the extract of the denuder filter compared to a standard HiVol filter and to determine the lifetime of the denuder bundle. The primary purpose of the program was to identify the chemical species present on the protected versus standard filter and thus determine the effect, if any, of the denuder.

### Summary of Experimental Work Program

- The removal efficiencies for $NO_2$ and $HNO_3$ by the denuder bundle were determined.
- The lifetime of the denuder bundle, based on the removal efficiency of ozone, was measured during tests of 4 and 6 weeks duration.
- In five warm weather field tests, filters protected by the denuder and unprotected filters, were obtained under selected meteorological conditions that were likely to place the samples downwind of potential PAH sources.
- Dynamic and static laboratory tests in which paired filters (unprotected and denuder-protected) were exposed to known $O_3$, $HNO_3$ or $NO_2$ concentrations were conducted.

## MATERIALS AND METHODS

### Field Sampling Methods

Four HiVol samplers were used, 2 standard HiVols and 2 denuder-HiVols. All samplers were equipped with electronic flow controllers that maintained a nominal flow of 40 CFM. Flowrates were set initially and checked at the start and end of each field test using a calibrated rotameter. The denuder HiVols were constructed in a fashion similar to that reported in the previous study (CSC, 1984), with minor changes to prevent the bundles from deforming and to allow easier removal of the housing from the HiVol during filter changing. Sampling was performed in Hamilton.

Concurrent ambient levels of particulate nitrate and gaseous nitric acid were measured at the site using parallel 47 mm diameter Teflon filter packs containing a Zefluor 1.0 μm Teflon filter backed up by two Nylasorb filters, at a flow rate of 4.9 L/min. Ambient ozone concentration data during the sampling runs were obtained from the OME station at Barton-Wentworth.

## Laboratory Determination of $NO_2$ and $HNO_3$ Removal Efficiencies of the Denuder

The effectiveness of the denuder for removing $HNO_3$ and $NO_2$ was examined by performing experiments using $HNO_3$ and $NO_2$ concentrations as well as relative humidity and temperature levels that were representative of ambient sampling conditions. For the $HNO_3$ and $NO_2$ removal experiments, the flow rate through the denuder tube was set as close as possible to the corresponding rate through a single tube (1 Lpm) in the HiVol bundle operating at 40 CFM. The nitric acid removal experiments were conducted at room temperature (~22-24°C) under average (40%) humidity conditions, and at 0°C (100% RH). The apparatus used to perform the experiments (shown in Figure 1) is similar to that used previously (CSC, 1984). Nitric acid concentrations before and after the denuder were measured by ion chromatographic analysis of the extract from nylon fiters placed in the sampling lines.

For the $NO_2$ removal experiments, a similar apparatus was used.

The $NO_2$ removal efficiency was determined by difference from measurements of the real-time $NO_x$ levels (Monitor Labs 8840) in the inlet and outlet streams.

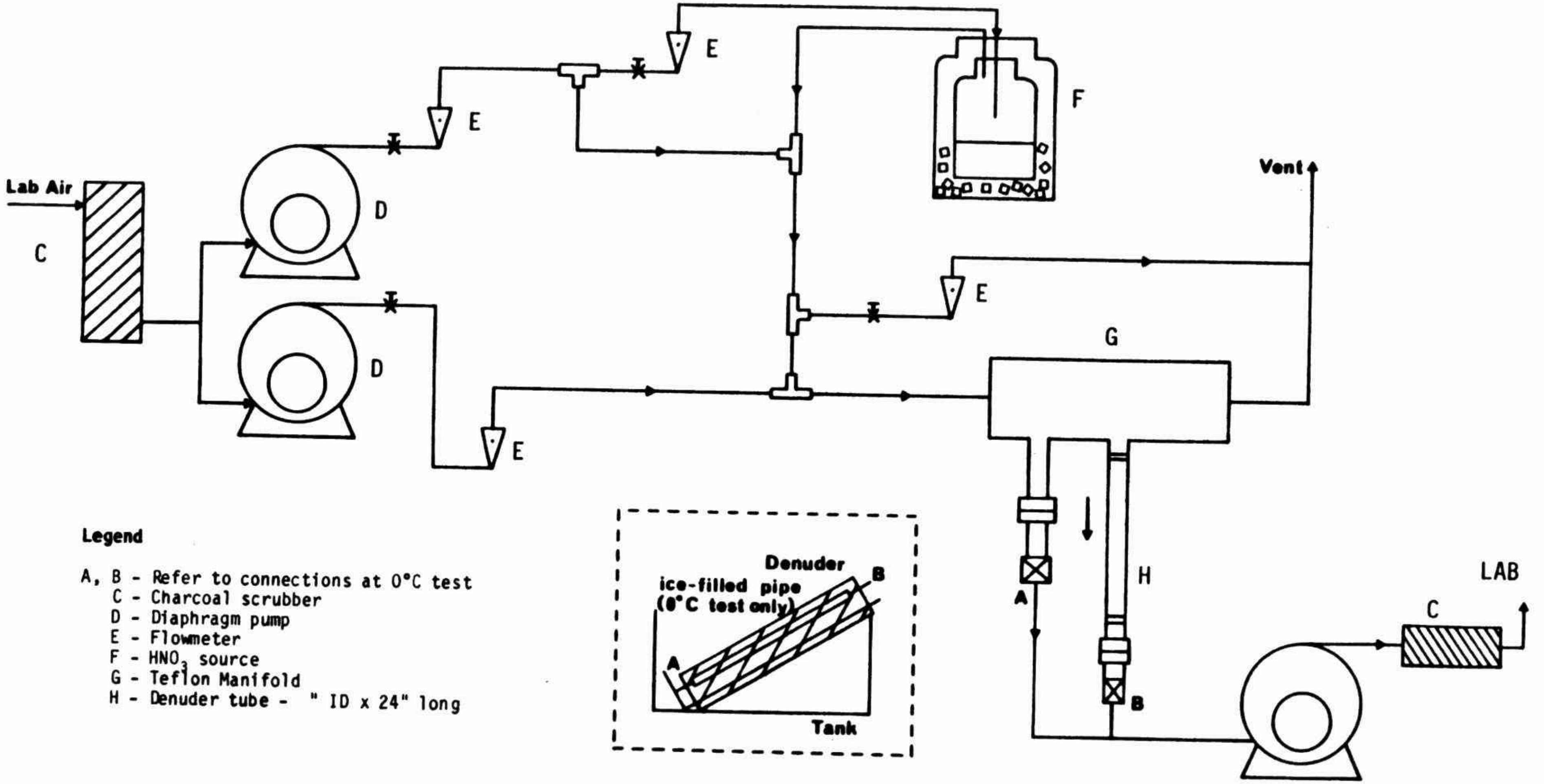
## Denuder Tube Lifetime Tests

A denuder HiVol sampler with a fresh denuder bundle was set up on the roof at Concord's head office. After 4 and 6 weeks sampling (not necessarily continuous) at 40 CFM, a different removable tube from the bundle was checked for ozone removal efficiency in the laboratory. A MEC 1000 ozone monitor was used to measure the ozone concentrations entering and leaving the denuder tube.

Ozone and meteorological data (RH, temperature, rainfall only) for the test duration were obtained from OME and the Canadian Climate Centre, for the nearest air quality and meteorological stations. The ozone data allowed calculation of the dose to which then the denuder bundle was exposed.

FIGURE 1

Apparatus for $HNO_3$ Removal Experiment



**Legend**

A, B - Refer to connections at 0°C test
C - Charcoal scrubber
D - Diaphragm pump
E - Flowmeter
F - $HNO_3$ source
G - Teflon Manifold
H - Denuder tube - " ID x 24" long

## Laboratory Static and Dynamic Exposure Tests

### Sample Collection

HiVol filters were preloaded with particulate matter from ambient sampling. The preloaded filters were exposed to $HNO_3$, $O_3$ and $NO_2$ in a series of laboratory experiments in order to provide exposure conditions that would facilitate identification and analysis of nitro- and/or oxy-PAH products, and also to test the effectiveness of the denuder. The conditions used would therefore of allow extrapolations to indicate what leves of oxy- and nitro-PAH derivatives may be found under ambient conditions.

### Static Exposure

Preloaded filters were exposed for 24 hours in a 175 L capacity polyethylene chamber (740 cm x 64 cm x 36 cm) lined with Teflon sheet.

### Static Exposures to $HNO_3$, $NO_2$ and $O_3$

A nitric acid vapour concentration (72 ppb) was established in the chamber by allowing a dish (20 cm diameter) containing 25% $HNO_3$ to equilibrate inside the chamber. $NO_2$ from a compressed gas cylinder (3.70 ppm $NO_2$ in air) was passed through the chamber filter. The $NO_2$ concentration was monitored using a Monitor Labs 8840 $NO_X$ analyser. An ozone generator was used to provide an ozone supply that was diluted with clean air from a compressor. The ozone concentration (250 ppb) was monitored with an MEC1000 ozone monitor.

### Dynamic Exposure

Sections of the preloaded filters were exposed to ozone or nitric acid containing air streams. During the exposures, a second section from the same filter was exposed to a reduced concentration by using laboratory scale denuder bundle (CSC, 1984) to remove the challenge gas. Table 1 summarizes the conditions used in dynamic exposure experiments.

## TABLE 1

### Summary of Conditions Used for the Dynamic Exposure of Preloaded HiVol Filters

| Filtered Code | Exposure Conditions | Concentration (ppb) | Time (min) | Flow Rate (lpm) | Volume ($m^3$) |
|---|---|---|---|---|---|
| F9 | $O_3$ | 200 | 1440 | 15 | 21.6 |
| F10 | | 200 | 1440 | 15 | 21.6 |
| F11 | | 190 | 1565 | 15 | 23.48 |
| F12 | | 200 | 1440 | 15 | 21.6 |
| F13 | | 200 | 1440 | 15 | 21.6 |
| F14 | | **200→500 | 1440 | 15 | 21.6 |
| F15 | | 200 | 1650 | 15 | 24.75 |
| F16 | | 190 | 1500 | 15 | 22.5 |
| DF9 | $O_3$ Denuder | *30→80 | 1440 | 15 | 21.6 |
| DF10 | | *15→35 | 1440 | 15 | 21.6 |
| DF11 | | *25→65 | 1440 | 15 | 21.6 |
| DF12 | | *35→75 | 1565 | 15 | 23.48 |
| DF13 | | *20→65 | 1440 | 15 | 21.6 |
| DF14 | | *10→25 | 1440 | 15 | 21.6 |
| DF15 | | *10→50 | 1650 | 15 | 24.75 |
| DF16 | | *25→45 | 1500 | 15 | 22.5 |
| F17 | $HNO_3$ | 70 | 1380 | 15 | 20.7 |
| F18 | | 60.5 | 1440 | 15 | 21.6 |
| F19 | | 80 | 1440 | 15 | 21.6 |
| F20 | | 43 | 1800 | 15 | 27.0 |
| DF17 | $HNO_3$ Denuder | 1.2 | 1380 | 15 | 20.7 |
| DF18 | | 7.5 | 1440 | 15 | 21.6 |
| DF19 | | 1 | 1440 | 15 | 21.6 |
| DF20 | | 1.2 | 1800 | 15 | 27.0 |
| CF21 | Control | 0 | 0 | 0 | 0 |

* Concentration at start of run.
** Malfunction caused ozone concentration to rise during run.

## Analytical Methodology

### Clean-up and Analysis of HiVol Filter Extracts From Field Sampling

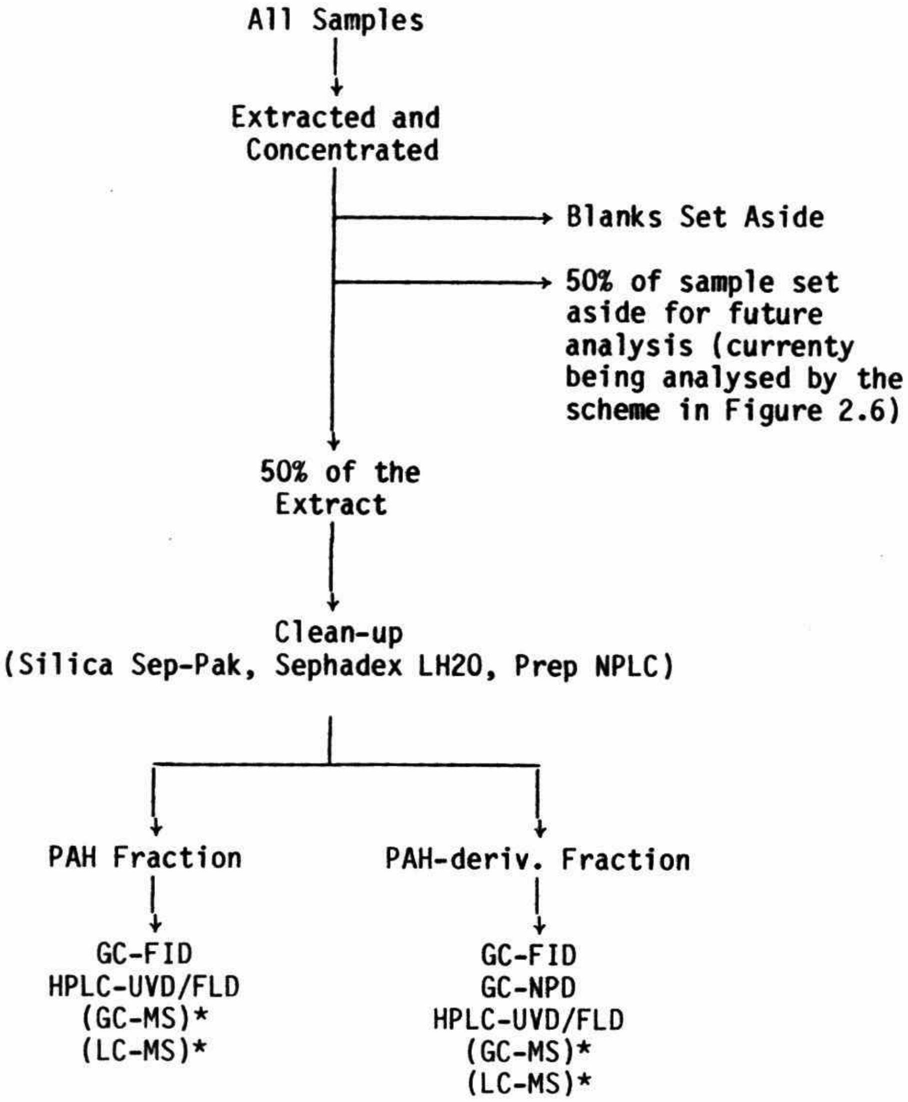
Methylene cloride extracts of the HiVol filters were processed according to a fractionation scheme developed by McMaster University (Quilliam et al., 1984) to give PAH and PAH derivative (e.g., oxy- and nitro-) fractions. GC-FID and/or HPLC analyses were performed on all samples for semi-quantitative analysis and fingerprinting of extracts. GC-MS (or LC-MS) were planned only for those samples representing different samplers, e.g., denuder versus non-denuder, that had significantly differing fingerprints. Pairs of samples were always taken through clean-up and analysis together. Figure 2 depicts the analytical scheme employed. Since there were no apparent differences in the GC and HPLC traces, LC-MS analyses were not done on all samples. One pair of fractions was analysed by LC-MS but no significant differences were observed under the conditions used.

### Extraction, Clean-up and Analysis of Static and Dynamic Exposure Experiments

Filters from the static and dynamic experiments were extracted individually (Soxhlet extraction) in a manner similar to that used in the field experiments. The fractionation scheme used for the static and dynamic samples is illustrated in Figure 3. This scheme represents an improvement over the earlier fractionation and analysis scheme in Figure 2 since it provides better precision in the measurement of relative levels of PAH and PAH derivatives. The clean-up scheme was designed to isolate aromatics in the samples from aliphatic and very polar compounds that interfere with the determination. The aromatics from the static experiments samples were analyzed by normal phase liquid chromatography with UV absorption detection (NPLC-UVD) and gas chromatography-mass spectrometry (GC-MS). The aromatics from the dynamic experiment samples were analyzed by NPLC-UVD and normal phase liquid chromatography-mass spectrometry (NPLC-MS). There was not sufficient material in these samples for replicate analyses or for GC-MS analyses.

FIGURE 2

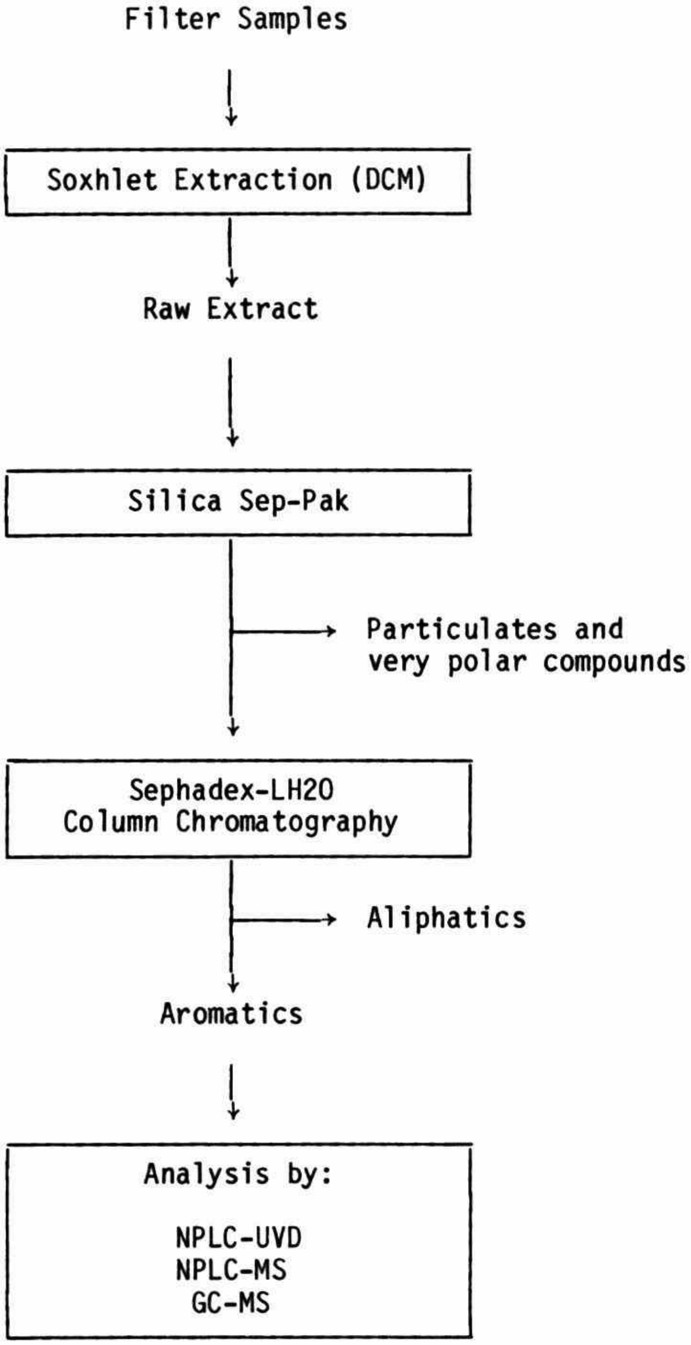
**Analytical Scheme for Field Samples**


All Samples
Extracted and Concentrated
Blanks Set Aside
50% of sample set aside for future analysis (currenty being analysed by the scheme in Figure 2.6)
50% of the Extract
Clean-up
(Silica Sep-Pak, Sephadex LH20, Prep NPLC)
PAH Fraction
PAH-deriv. Fraction
GC-FID
HPLC-UVD/FLD
(GC-MS)*
(LC-MS)*
GC-FID
GC-NPD
HPLC-UVD/FLD
(GC-MS)*
(LC-MS)*

(* Sample set for GC-MS and LC-MS chosen on basis of samples showing greatest differences in GC or HPLC traces)

FIGURE 3

**Fractionation and Analysis Scheme for Filters From Static and Dynamic Experiments**



## RESULTS AND DISCUSSION

### Denuder Effectiveness For Removing $HNO_3$ and $NO_2$

#### $HNO_3$ Removal Experiments

Laboratory tests performed to determine the denuder's effectiveness is removing $HNO_3$ are summarized in Table 2. Included in Table 2 are the $HNO_3$ exposure conditions (challenge concentration and duration of exposure) and the nitric and concentration after passage through the denuder (Breakthrough [$HNO_3$] column in Table 2). The room temperature results showed only marginal breakthrough even though the $HNO_3$ concentration was 490 $\mu g/m^3$ or ~190 ppb; about one order of magnitude greater than what might be found in polluted atmospheres. Summing the exposures (duration x concentration) in each phase of the test up to breakthrough, indicated a life of 16,500 ppb-hours. This is probably a lower limit since a very elevated concentration was used for the last phase. Assuming 10 ppb ambient $HNO_3$ concentration, the denuder would be effective for a time span of 10 weeks.

#### $NO_2$ Removal Experiments

Two removal efficiency experiments were conducted, one at ~45% RH and 22°C and the other at ~100% RH and 23°C. Test duration was 24 hours in each case and a fresh denuder tube was used. For the lower humidity test, removal efficiency after 24 hours was only 15% at 72 ppb $NO_2$, while for the higher humidity test, removal efficiency was 31% at 61 ppb $NO_2$. Removal efficiency in both cases was only marginally better at the start of the experiment. Since room temperature tests showed that the $NO_2$ removal efficiencies were poor, it was decided not to pursue tests at 0°C. Any denuder in field use would be required to be effective throughout the entire range of ambient temperatures.

### Denuder Lifetime Tests for Ozone Removal Effectiveness

The denuder tube removed after four weeks and six exposure were tested in the laboratory at 23°C and 65% RH for ozone removal efficiency at various challenge ozone concentrations. The test showed removal efficiencies of:

TABLE 2

## $HNO_3$ Removal Experiments

| Experiment | Conditions | $HNO_3$ Exposure | Breakthrough [$HNO_3$] | Efficiency of Phase |
|---|---|---|---|---|
| 1 | 23-25°C | Phase 1: after 18.25 hours, 269 $\mu g/m^3$ | 0 | 100% |
| | ~40-50% RH | Phase 2: 18 - 42.25 hours, 134 $\mu g/m^3$ | <0.7 $\mu g/m^3$ | ~100% |
| | 0.96 LPM denuder flow | Phase 3: 42.25 - 112.1 hours, 490 $\mu g/m^3$ | 3.2 $\mu g/m^3$ | 99.3% |
| | | $\sum$ Phases ≡ 16,500 ppb-hours | | |
| 2 | ~0°C | Phase 1: after 20 hours, 20.0 $\mu g/m^3$ | 3.1 $\mu g/m^3$* | 85% |
| | ~100% RH | Phase 2: after 41 hours, 19.1 $\mu g/m^3$ | 0 | 100% |
| | 0.96 LPM | $\sum$ Phases = 310 ppb-hours | | |

* since Phase 2 showed no breakthrough this result is surprising

25 ppb ≡ 64 $\mu g/m^3$ at 25°C and 101.3 kPa

After 4 weeks

- 100% at 5 ppb ozone
- 80% at 25 ppb
- 65% at 160 ppb
- 60% at 210 ppb

New Tube

- 100% at 155 ppb

After 6 weeks

- 90% at 50 ppb
- 85% at 110 ppb
- 85% at 155 ppb

New Tubes

- 100% at 200 ppb

Estimates of the ozone dose to the denuders (the product of the test duration and the mean ambient ozone concentration) for periods 1 and 2 were respectively 98 and 132 ppb-weeks. On the basis of two data points it is not feasible to establish a correlation between dose and efficiency. However, the data do establish that even after 6 weeks continuous operation, the denuder remained effective (65 to 80% removal efficiency) when challenged with ozone concentrations of ~150 ppb.

**Analytical Results - Field Runs**

Examination of the chromatograms showed that only one field run S3 showed minor differences between the standard and denuder sampler systems. Several PAH compounds were identified in the PAH fraction from the standard HiVol and the denuder HiVol filter extracts in run S2. Identification was by comparison of UV-visible spectra of the HPLC fractions with that of standards. The reproducibility of most analyses between duplicate samples was sufficiently good to suggest that any significant difference would have been observed readily.

The data were somewhat surprising, i.e., there appeared to be little impact of the denuder. There are three possible explanations for the lack of difference:

- the denuder was not effective,
- there was little artifact formation occurring in the particulates during sampling,
- any artifact formation had already occurred in the atmosphere.

In view of this the static and dynamic laboratory exposures of precollected samples were conducted.

## Static and Dynamic Laboratory Exposure Tests

All extracts from similarly treated filters were pooled, eight samples were generated, namely, 4 from the static experiments (from control, ozone nitric acid and nitrogen dioxide exposed filters) and 4 from the dynamic experiments (standard and denuder protected from nitric acid and ozone exposure).

The aromatics from the static experiment samples were analyzed by normal phase liquid chromatography with a UV absorption detector (NPLC-UVD) and gas chromatography-mass spectrometry (GC-MS) using the cleanup scheme shown in Figure 3. The aromatics from the dynamic experiment samples were analyzed by NPLC-UVD and normal phase liquid chromatography-mass spectrometry (NPLC-MS). There was not sufficient material in these samples for replicate analyses or for GC-MS analyses.
The data from the NPLC-MS analyses were examined by plotting the mass chromatograms of various PAH and PAH derivatives, normalized with respect to that of 276-PAH which acted as an internal standard to correct for volumetric errors and which was assumed not to undergo transformation (or transformation to similar extents) when unprotected by the denuder. Peak areas from the mass chromatograms of the standard and denuder samples were incorporated in the following equation for a "noramlized ratio".

Normalized Ratio =

$$\left(\frac{\text{Area of M of standard HiVol}}{\text{Area of M of denuder HiVol}}\right) \times \left(\frac{\text{Area of M 276 peak from denuder HiVol}}{\text{Area of M 276 peak from standard HiVol}}\right)$$

If there were no differences between the standard and denuder samples, this ratio should be 1.00. However, if there were certain PAH converted to PAH derivatives, the ratio for the PAH would be less than 1.00, while that of the derivatives would be greater than 1.00.

## Static Experiments

There were no apparent differences in the NPLC-UVD or GC-MS traces between the control sample extract and the extracts from the filters exposed to ozone, nitric acid or $NO_2$. It is likely that the dose (concentration x exposure duration) was insufficient to cause discernable reactions of the filters.

## Dynamic Experiments

Analytical data for the four pooled samples are given in Table 4. Peak areas for identified PAH and ketone as well as quionone PAH derivatives in the standard (STD) and denuder protected (DENUD) filter extracts are listed for the ozone and nitric acid experiments. The normalized ratios for the standard to the denuder-protected peak areas for each compound are also indicated.

The effectiveness of the denuder will be indicated by

a) the appearance of different species in the standard versus the denuder protected samples, and/or

b) differences in the amounts of PAH derivatives and/or PAH compounds.

## Ozone Exposure

The NPLC-UVD analyses showed that there were no major differences in the standard and denuder-protected samples, i.e., no new compounds (extra peaks) appeared in the standard versus the denuder-protected chromatograms. Dramatic differences were observed in the NPLC-MS data of the ozone samples. Compounds in the PAH derivative region showed normalized ratio values significantly greater than 1.00 (2 to 10), which means they were at a higher concentration in the standard sample versus the denuder sample. The mass spectra of these PAH derivaties show losses of 28 mass units from the molecular ion, which indicated that they are ketone and quinone derivatives of PAH. Positive identification of these compounds was not possible.

The data in Table 4 also show that the concentrations of some PAH are significantly lower in the ozone standard sample, which could be the result of reaction of these PAH with ozone to give the observed PAH-derivatives and other undetected products. It is useful to note that

## TABLE 4

## Analytical Results From LC/MS Analyses of Pooled Dynamic Samples

| Compound Identity | M/Z | Scan # | Ozone Dynamic Peak Area STD | Ozone Dynamic Peak Area DENUD | Norm.* Ratio | Nitric Acid Dynamic Peak Area STD | Nitric Acid Dynamic Peak Area DENUD | Norm. Ratio |
|---|---|---|---|---|---|---|---|---|
| Phenanthrene | 178 | 354 | 3859 | 20898 | .4 | 25094 | 30590 | .7 |
| Fluoranthene | 202 | 371 | 3846 | 15341 | .5 | 28140 | 25788 | .9 |
| Pyrene | 202 | 378 | 5911 | 23824 | .5 | 37301 | ? | |
| Benzofluorenes | 216 | 378 | 706 | 4340 | .4 | ? | ? | |
| 264-PAH | 264 | 379 | 194 | 786 | .6 | ? | ? | |
| 266-PAH | 226 | 389 | 1773 | 6475 | .6 | 24101 | ? | |
| 240-PAH | 240 | 397 | 796 | 2135 | .8 | ? | ? | |
| 265-PAH | 264 | 397 | 542 | 1052 | 1.2 | ? | ? | |
| Benz(a)anthracene | 228 | 398 | 7864 | 25639 | .6 | 66726 | 62832 | .9 |
| Chrysene | 228 | 402 | 1987 | 5605 | .7 | 17850 | 18226 | .8 |
| 240-PAH | 240 | 402 | 683 | 1980 | .8 | ? | ? | |
| 252-PAH | 252 | 413 | 35443 | 77576 | 1.0 | 140710 | 153793 | .7 |
| 264-PAH | 264 | 415 | 1480 | 3068 | 1.1 | ? | ? | |
| 276-PAH | 276 | 427 | 3342 | 75267 | 1.0* | 176837 | 163050 | 1.0* |
| 278-PAH | 278 | 429 | 7464 | 14301 | 1.1 | 27965 | 31214 | .7 |
| Anthraquinone | 208 | 577 | 1929 | ? | | 14339 | 14374 | .8 |
| ? | 226 | 677 | 4438 | 2229 | 4.4 | 9571 | 7221 | 1.1 |
| 230-PAK | 230 | 500 | 4055 | 5364 | 1.7 | 33364 | 39760 | .7 |
| 230-PAK | 230 | 534 | 1080 | 1260 | 1.9 | 3952 | 4343 | .7 |
| 230-PAK | 230 | 618 | 7628 | 5862 | 2.9 | 41641 | 48755 | .7 |
| 230-PAK | 230 | 648 | 7531 | 5649 | 3.0 | 33061 | 30413 | .9 |
| 254-PAK | 254 | 508 | 746 | 1323 | 1.2 | 45504 | 4077 | .9 |
| 254-PAK | 254 | 586 | 2862 | ? | | 7534 | 6862 | .9 |
| 254-PAk | 254 | 608 | 3365 | 2824 | 2.6 | 8396 | 8952 | .8 |
| 254-PAK | 254 | 638 | 1433 | 644 | 5.0 | ? | ? | |
| 254-PAK | 254 | 677 | 14787 | 9821 | 3.3 | 37052 | 29983 | 1.0 |
| 258-PAQ | 258 | 536 | 3556 | 3394 | 2.3 | 12671 | 13664 | .8 |
| 258-PAQ | 258 | 600 | 2357 | 546 | 9.7 | 6924 | 7584 | .7 |
| 278-PAK | 278 | 606 | 5458 | 4398 | 2.7 | 13130 | 13128 | .8 |
| 278-PAK | 278 | 628 | 1197 | 412 | 6.5 | 2686 | 2558 | .9 |
| 282-PAQ | 282 | 639 | 3333 | 1381 | 5.4 | 9420 | 9280 | .8 |
| 1-Nitropyrene | 247 | 465 | 2234 | 3189 | 1.5 | 3288 | 3505 | .8 |
| Nitrofluoranthene | 247 | 476 | 499 | 1064 | 1.0 | 2623 | 3726 | .6 |

* The 276-PAH peak was used as an internal standard to derive the normalized ratio = $(Asj/Adj)x(Ad_{276}/As_{276})$, where A = area, s = standard hivol, d = denuder hivol, j = peak of interest, and 276 = 276 PAH.

the compounds with saturated carbons, such as the benzofluorenes (MW 216), are most susceptible to oxidation, and have low ratios though not exclusively so. The low normalized ratio for the PAH compounds could be due to evaporative losses, but this can be ruled out since both samples were subjected to similar airflows, both during initial sample collection and during the dynamic exposure tests. In addition, care was taken to ensure that sample pairs were treated similarly during the cleanup procedures so that evaporative losses were minimised.

## Nitric Acid Exposure

NPLC-UVD and NPLC-MS data did not indicate differences between the standard and the denuder-protected samples in the dynamic nitric acid exposure experiments. The levels of the oxy-and nitro-PAH were similar in both types of samples.

These results point to one or more of the following possibilities

a) the dynamic exposure conditions were inappropriate to effect nitration reactions

b) the reactions did occur, but the denuder afforded no additional protection

c) nitration reactions occurred prior to the dynamic exposure i.e., during sample collection

Examination of Table 4 shows that while the levels of the 1-nitropyrene were similar in the nitric acid exposure runs, the 1-nitropyrene level in denuder-protected sample of the ozone run was surprisingly higher than that for the standard HiVol. The converse was true for nitrofluoranthene i.e., similar levels for standard and denuder protected samples in the ozone experiments but higher levels for the denuder protected samples in the nitric acid experiments. The explanations for these results are not clear and on the basis of two pairs of samples, speculation about likely explanations are inappropriate.

## SUMMARY AND CONCLUSIONS

The experimental data from this study have shown the following:

1. The denuder is effective not only for ozone, but also for nitric acid. Breakthrough of $HNO_3$ was found to occur only after 16,500 ppb-h exposure. Assuming ambient nitric acid concentrations of 10 ppb, the estimated lifetime for nitric acid would be 1650 h or ~70 d continuous operation. Within these limits, the removal efficiency for $HNO_3$ was >99% at room temperature and >85% at 0°C.

2. After 42 days continuous field operation with a mean ozone concentration of 22 ppb, equivalent to an ozone dose of 23760 ppb-h, the denuder's efficiency for removing ozone was 85% for a laboratory ozone challenge concentration of 155 ppb and 90% removal efficiency for 50 ppb.

3. Analysis of extracts from standard and denuder HiVol field samples showed insignificant differences in the PAH derivative composition. The concurrent ozone and nitric acid concentrations during the field runs were not exceptionally high ($O_3$ daily maximum in the range of 40-66 ppb and 24h average $HNO_3$ 2-5 ppb). The $O_3$ and $HNO_3$ levels however are typical of the majority of non-episodic days.

4. Laboratory tests showed that the denuder reduced the formation of oxygenated PAH derivatives within fairly broad analytical uncertainty. Ozone concentrations to which standard, i.e., unprotected, filters were exposed were ~200 ppb.

5. Dynamic laboratory tests in which preloaded filters were challenged with nitric acid - in one case directly and in the other case protected by the denuder, showed no differences in the PAH derivative fractions between samples.

6. Analogous tests on filters using static exposure conditions for $O_3$, $HNO_3$ and $NO_2$ separately showed no differences in the PAH derivative fraction compared to control filters.

The apparent lack of reaction with nitric acid is surprising but conflicting results regarding the nitration of PAH are found in the literature.

Although several investigators have studied the reactions of PAH with potential nitrating agents such as NO (Hughes et al., 1979), $HNO_2$ (Lindskog, 1983), $NO_2$ (Grosjean et al., 1983; Pitts et al., 1978) and $N_2O_5$ (Pitts et al., 1985), the most likely nitrating agents appear to be $N_2O_5$ (Pitts et al., 185) or $HNO_3$ (Grosjean et al., 1983). Conclusive evidence regarding the extent to which nitration of PAH in ambient sampling remains to be demonstrated.

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# A PARTICLE-SIZE SPECIFIC ELEMENTAL MASS BALANCE FOR APPORTIONMENT OF AMBIENT AEROSOL SAMPLED WITH MULTISTAGE FRACTIONATORS

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## ABSTRACT

Photon and thermal neutron activation analysis were used to determine the concentrations of up to thirty elements in aerosol samples collected by cascade impactor in Toronto, Canada during the period July to December of 1985. Examination of the particle-size distributions thus obtained led to the development of a new receptor model. A size-specific elemental mass balance was used to apportion the mean ambient Pb aerosol concentration amongst four contributing sources as follows: secondary lead refinery emissions (46%), refuse incineration (28%), automotive exhaust (25%) and re-entrained soil and dust (<1%). The predicted concentrations of Al, As, Br, Na, Pb, Sb, and Zn agreed with observed concentrations to within a factor of two for most elements in most particle size fractions.

## INTRODUCTION

Increased use has been made during the last decade of receptor models to identify aerosol sources and to apportion ambient particulate matter among a set of contributing sources. /1,2/ Such models utilize as input data a set of aerosol measurements made at a particular sampling location (receptor site) and include as parameters, some or all of: chemical composition, particle-size distribution, site location and meterological data, in attempts to resolve statistically the major emission sources contributing to ambient particulate matter at that site. This method is considered less capital-, manpower- and time-intensive than alternative, much-used approaches of coupling aerosol emission inventories with dispersion models to achieve similar results.

In a previous study, Pringle and Jervis /3/ performed factor analysis upon a data set containing the concentrations of 15 chemical elements in 18 airborne particulate matter samples and were able to identify the primary aerosol sources at a site in Port Credit, a relatively non-industrial satellite town of Toronto, Canada. Five emission sources contributing at that location were labelled as: soil, transportation vehicles, refuse and oil combustion, winter road salt and, one unidentified arsenic source, and these sources had been resolved through factor analysis and their relative contributions to the ambient aerosol determined through the use of a chemical mass balance (CMB) receptor model.

A major limitation of this method of source apportionment in highly industrialized areas is the inability of the CMB model to differentiate between aerosol sources which have similar chemical compositions (in terms of interelemental concentration 'profiles'), viz.refuse incinerators, automobile emissions and non-ferrous industries such as zinc and lead processors. To overcome some of these limitations in the methods, a modified approach has been used in the research presented here: the particle-size distributions of ambient particulate matter are measured and differences in size distribution, for certain elements among emission sources, are utilized to separate 'elementally-collinear' sources.

Whereas several (other) published studies /4,5,6/ have indirectly made use of particle-size data by separating the aerosol into coarse and fine fractions (<2.5 µm, 2.5-10 µm), which data is then used separately in receptor modelling, the objective of the present study was to divide the aerosol into multiple size fractions so that unique aerosol size-distributions were not lost by separation, during sampling, only into two fractions.

There are several requirements of the analytical procedures that are chosen for use in receptor studies: the technique chosen must be able both to to determine as many of the chemical elements as possible in particulate samples small in mass and also be capable of determining many of the 'key ' marker' elements which help distinguish different sources contributing to the aerosol collected at the receptor site. Furthermore, because of the statistical nature of the modelling procedures, variance in the experimental results due to the analytical techniques themselves, must be minimized. A sensitive multielemental instrumental method such as activation analysis is ideally suited to meet these requirements; in this work, a complementary coupling of thermal neutron and photonuclear activation techniques allowed determination of most elements of interest as well as permitting two independent determinations for a subset of elements as a means of data quality control./5/ Further, in this study, when individual aerosol size fractions contained as little as one tenth of the total mass of particulates collected, the use of the activation technique was even more essential as a means to obtain the necessary analytical results for practical modelling.

## MATERIALS and METHODS

A series of samples of airborne particulate matter was collected at regular intervals during the period July-December, 1985 using Anderson and Sierra high-volume cascade impactors deployed in central Toronto, Canada. The samplers were operated for 12 hour periods at a regulated flow-rate of 0.019 $m^3s^{-1}$ (or, 40 scfm) and filtered a total air volume of approximately 820 $m^3$. By means of the Sierra 235 and Anderson Hi-vol impactor heads, the airborne particulate was separated during collection into 5 or 6 size fractions according to their aerodynamic diameters. Scanning electron microscopy (SEM) and elemental analyses performed on typical aerosol specimens indicated that large particle 'bounce-off' from upper stages - a phenomenon at question with some cascade impactors - was less pronounced for the Sierra 235 impactor than for the Anderson instrument. A prior soaking of the collection substrates in a Vaseline/hexane (1:10) solution, repeated several times, further improved particle retention on the impactor upper stages so that the particle-size distribution determined by direct examination with SEM was found to be quite close to the manufacturer's specified stage particle-size cutoffs, viz. 7.2, 3, 1.5,0.95 and 0.49 µm at 50% collection efficency, with a 'backup' filter used to collect all remaining particles of lower diameter in the incident airstream. Whatman # 41 cellulose filter material was chosen as the collection medium both for the substrates in the several impactor stages and for the 'backup' filters because of its low trace element content and its structural integrity when moist. Glass fibre material, the standard aerosol filter medium, was considered particularly unsuitable as a substrate for activation analysis because of high Na and ash content.

After particulate sample collection, each substrate was halved and the edges trimmed to remove any contamination from handling or contact with the sampler. A half section of each was analyzed by photonuclear activation analysis(IPAA) at the National Research Council (Canada) electron linear accelerator facility (LINAC) in Ottawa. The remaining half section was subjected to neutron activation analysis (INAA) at the University of Toronto SLOWPOKE-2 nuclear reactor facility. Backup filters were divided into four sections, trim-

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[1] Sierra 235 and Anderson Hi-vol impactor heads are products of Anderson Samplers Inc.

Table 1

MEAN ELEMENTAL CONCENTRATIONS OF AIRBORNE PARTICULATE MATTER (ng.m$^{-3}$)

| Element | Toronto (this study) | Toronto Suburb (1985)/3/ | Washington (Kowalczyk et al,1982)/8/ | Chicago (Scheff et al,1984)/4/ |
|---|---|---|---|---|
| Al | 1100 ± 420 | 600 | 1350 ± 110 | 1630 ± 900 |
| As | 4.6 ± 6.7 | 5.3 | 3.25 ± 0.2 | 4.3 ± 4.7 |
| Br | 61 ± 28 | 70 | 136 ± 9 | - |
| Ca | 4150 ± 1780 | 2150 | 860 ± 40 | 3420 ± 3290 |
| Fe | 1150 ± 670 | 640 | 1000 ± 60 | 2310 ± 1530 |
| Mn | 49 ± 28 | 34 | 17 ± 2 | 89 ± 71 |
| Na | 850 ± 540 | 1520 | 300 ± 20 | 2540 ± 2480 |
| Pb | 1100 ± 1510 | 300 | 440 ± 20 | 490 ± 230 |
| Sb | 11.3 ± 17 | - | 2.1 ± 0.2 | - |
| Sc | 0.2 ± 0.08 | 0.13 | 0.33 ± 0.03 | - |
| Ti | 100 ± 60 | 64 | 110 ± 10 | - |
| V | 3.8 ± 2.9 | 5.9 | 25 ± 2 | 8.74 ± 6.39 |
| Zn | 640 ± 1040 | 108 | 85 ± 6 | 304 ± 288 |
| n(no.of samples) | 8 | 18 | 130 | 35 |

med and two sections each were subjected separately to (duplicate) IPAA and INAA. Complete details on the experimental procedures, irradiation parameters, accuracy and interferences relevant to the analysis of particulate matter and similar materials by IPAA and INAA have been reported previously. /3,7/ By use of a 35 MeV electron energy and typical range of beam currents of 30-60 µA, Pb,Zr,Ca,Ti,Sb and As could be determined, of which Pb, Zr and Ti were not measured with acceptible precision by INAA; other elements among these were used for accuracy checks between the two techniques.

The other sections of each particulate sample were then subjected to sequential thermal neutron irradiations for periods ranging from 2 min. to 16 h, for determination using short-lived nuclides of: Al,Ca,Cl,Cu,I,In,Mn,Ti,V; and using longer-lived nuclides: Ag,As,Br,Ce,Co,Cr,Cs,Eu,Fe,Hf,K,La,Sb,Sc,Sm,Th,W and Zn. Irradiations were at neutron fluxes from 2.5-10 x $10^{15}$ m$^{-2}$s$^{-1}$ and Ge-gamma-spectrometry performed at periods from 1 min. to 60 d.

A U.S. Nat. Bureau of Standards reference material coal, 1632a bituminous coal, was used as a multielemental standard both for IPAA and INAA. Since the samples and standards were quite different in volume and shape, care was taken to spread the standard to extend over a volume equal to that of the folded filter samples and this was accomplished by placing a known small quantity of the standard in each of several polyethylene capsules. Other standards: NBS air particulate reference material, SRM 1648 and flyash, SRM 1633a, were used also to verify analytical accuracy which was found to be, typically ±10% between reference materials.

## RESULTS and DISCUSSION

The mean concentrations of a selection of sixteen of the chemical elements measured in airborne particulate matter from Toronto, Canada during a mid-1985 sampling period are presented in Table 1. Although the concentrations of 30 elements were determined, those listed and included in the calculations presented here were considered to be the most useful for the purpose. A complete tabulation of results will be given in a forthcoming report. /8/ Presented also in the table for comparison are aerosol concentrations from recent studies

FIGURE 1

TYPICAL PARTICLE-SIZE DISTRIBUTIONS IN THE TORONTO AEROSOL

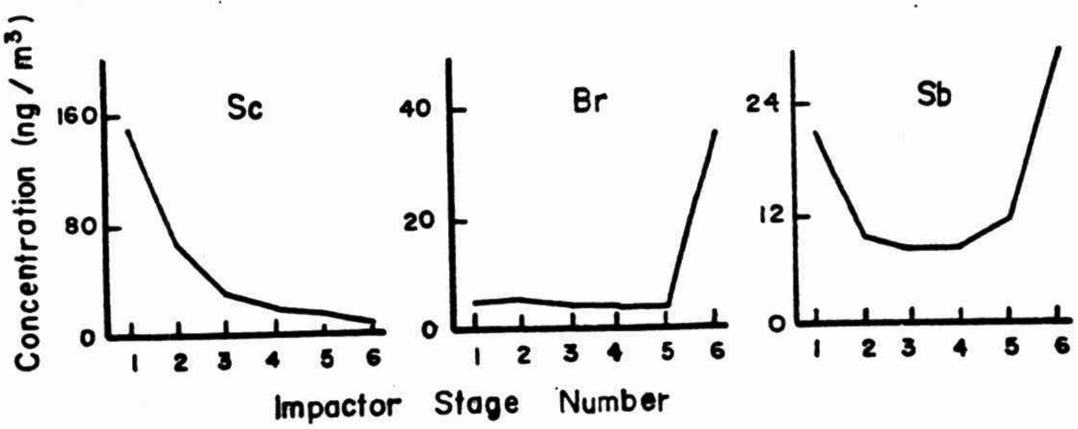
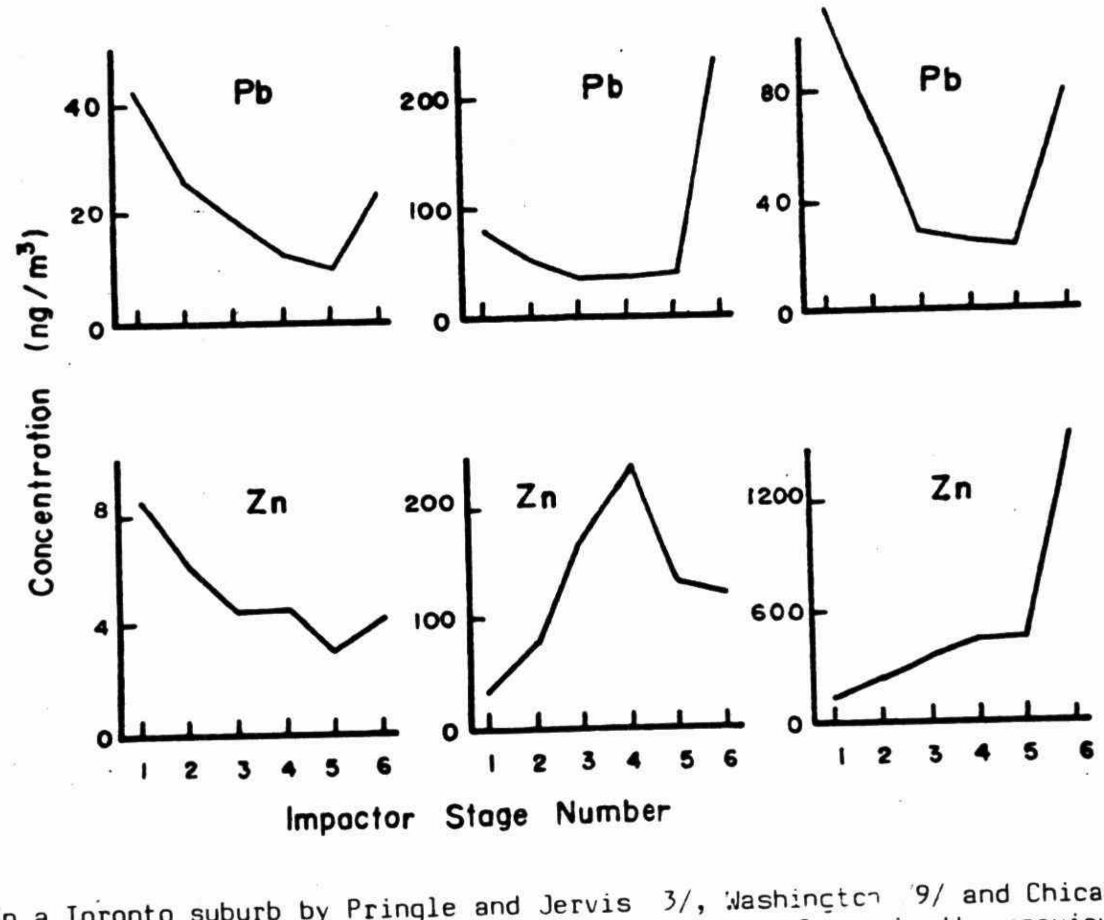


FIGURE 2

LEAD AND ZINC PARTICLE-SIZE DISTRIBUTIONS: INDIVIDUAL SAMPLES



in a Toronto suburb by Pringle and Jervis /3/, Washington /9/ and Chicago /4/.

Compared to the relatively-low concentrations found in the previous Toronto study /3/, the present results indicate levels of most elements which are similar to those reported for the two U.S. cities. However, the present measurements were made in a more industrialized area than the 1985 study and there was no snow cover to reduce soil entrainment, as there was in the earlier study. By way of comparison with relatively non-industrial Washington, most elemental concentrations are higher in this study although still less, in most cases, than those from Chicago, a much more heavily industrialized city.

It is assumed that the higher Ca values for Toronto were a result of a strong limestone component in local soils and rocks and that lower amounts of Br indicated a higher ratio of unleaded to leaded gas. The significantly higher concentrations of Pb, Sb and Zn were assumed to arise from local industry and the relatively large standard deviations for those elements to be caused by the coincidence of daily wind changes with a 12 hour sampling period which tended to reduce time-averaging of emissions from local point sources of aerosol.

## Size Distributions

As was the case for the previous Toronto study, all of the elemental size distributions were classified by their shape as belonging to one of three groups. Figure 1 illustrates this classification with elemental concentration plotted against impactor stage number; stages 1 to 6 represent effective particle cutoff diameters of 7.2, 3, 1.5, 0.95, 0.49 and <0.49 µm, respectively.

The elements K, Al, Ca, Fe, Sc, Ti and Th all had a particle-size distribution profile which consisted primarily of large particles, similar to the profile for scandium in Figure 1. This group of elements is commonly associated with soil particles /4,9,10/ and their relatively large particle size indicates a physical entrainment process. This size profile was therefore considered to be that of re-entrained soil and dust. In contrast, the elements Br and I, as well as Pb, Sb, Zn and Cl in a few samples, possess particle-size profiles which show a predominantly fine-particle bias. These elements are associated with combustion processes such as automotive exhaust (Br), refuse incineration (Zn, Pb, Sb) or coal combustion (I) and their profiles are indicative of combustion vapour phase emissions which have condensed on fine particles. /11/

Most of the remaining elements (As, Mn, V, Se, Na as well as Pb, Sb, Zn in some samples) have size distributions which resemble the Pb profile in figure 1 in that they show pronounced elevations in concentration for both small and large particles, indicating some combination of both soil entrainment and combustion as the aerosol source. The profiles for Zn, shown in Figure 2 are unusual since they indicate that there might be three different source mechanisms for the emission of zinc-bearing particles with a predominance of either of large, medium or small particles. It was felt that this type of difference could be exploited to separate the emissions from several sources of aerosol which have similar elemental compositions.

## Receptor Model Application

In the 1985 study of a Toronto suburb, two receptor models were used, factor analysis and a chemical mass balance (CMB). Both are based on equation (1) where the observed concentration of element i ($a_i$) is assumed to be the

$$a_i = \sum_j b_{ij}c_{ij} \tag{1}$$

linear sum of the contributions from j sources. In factor analysis, a matrix (A, n x i) of i elemental concentrations in n samples ($a_{in}$) is reduced to a matrix of j hypothetical vectors (B, i x j) such that a maximum of the variance in A is reproduced by the new matrix. The elements which are most strongly associated with each new vector ($b_j$) served to identify that vector, or "source", by their common associations. Thus the number and type of major aerosol sources contributing to the ambient aerosol can be determined with no a priori assumptions. With this information, the elemental composition of emissions from each source can be approximated ($c_j$) and, using least squares regression to solve equation 1 for i elements in each sample, the contribution of each

TABLE 2

**ELEMENTAL SOURCE PROFILES ( μg/g )**

| Element | Soil | Automotive Sources | Refuse | Refinery Emissions | Z1 | Z2 | Road Salt |
|---|---|---|---|---|---|---|---|
| Al | 50000 | 700 | 14000 | 3000 | 3600 | 36000 | 0 |
| As | 10 | 0 | 200 | 2600 | 120 | 120 | 0 |
| Br | 31 | 45500 | 1000 | 54 | 44 | 44 | 750 |
| Ca | 76000 | 1700 | 17000 | 4700 | 700 | 700 | 6000 |
| Fe | 30000 | 2000 | 6500 | 11000 | 4000 | 4000 | 0 |
| Mn | 650 | 1500 | 730 | 540 | 300 | 300 | 0 |
| Na | 26500 | 0 | 82000 | 10000 | 2200 | 2200 | 350000 |
| Pb | 200 | 130000 | 75000 | 160000 | 3400 | 3400 | 0 |
| Sb | 9 | 0 | 1500 | 7600 | 15 | 15 | 0 |
| V | 60 | 0 | 100 | 25 | 30 | 30 | 0 |
| Zn | 200 | 1000 | 120000 | 2400 | 150000 | 150000 | 0 |

source to the ambient airborne particulate matter ($c_j$) can be calculated.

It has been noted /4,12/ that receptor models are unable to reliably separate sources of similar elemental composition (collinear sources). In factor analysis a knowledge of possible contributing sources is necessary to realize that a pair of collinear sources exist since only one eigenvector will exist for that pair. In the CMB, either one elemental profile is used for both sources or the least-squares regression will cause one source to have a large positive contribution to the aerosol and the other to have a large negative contribution and so provide the best possible fit.

This is not a serious problem in a lightly industrialized city; the only pair of elementally collinear sources modelled by Kowalczyk et al /13/, in their study of aerosol in Washington, D.C., was coal flyash and soil and they were able to use Mn and As to help differentiate between the two sources. However, in the industrialized area where the present study was conducted there are many local industrial sources. For example, a secondary lead refinery, two municipal incinerators and two high traffic-volume roads all contribute to the Pb concentration in the ambient aerosol. Here, instead of the traditional approach mentioned above, the CMB was altered so that each elemental concentration (ai) in equation (1) was replaced by the six concentrations of that element in the six separate particle-size fractions ($a_i1$, $a_i2$,.... $a_i6$) collected by cascade impactor. In effect, the several sources which rely on a particular marker element, Pb in this case, can be separated by their respective particle-size profiles. In order to test this new model concept, an attempt was made to calculate the contributions of various sources to the ambient Pb aerosol. To reduce the number of assumptions required in the model, a subset of elemental and particlesize data was composed such that only marker elements and particle-size profiles for major sources of Pb were considered along with markers which strongly affect calculations for these sources.

Although factor analysis was employed to identify the major contributing sources of aerosol in the previous Toronto study, this was not overly useful in the present research since, where several sources have collinear composition profiles only the sources with with the largest concentration variations, would be identified. Variations in the lead refinery emissions were much greater in all size fractions than those for the other sources of Pb. Furthermore, only eight samples were analyzed for this study, too small a number for all but the grossly varying sources to be identified.

The composition of the data subset was determined by examination of the particle size profiles and a review of the literature to examine likely elemental profiles /2/. For example, from Figure 2 the lead aerosol size distribution

## TABLE 3

### PARTICLE-SIZE PROFILES FOR VARIOUS SOURCES (% OF TOTAL CONCENTRATION)

| Impactor Stage No. | ECD (μm) | Soil, Salt | Auto | Refuse, Z1 | Refinery Emissions | Z2 |
|---|---|---|---|---|---|---|
| 1 | 7.2 | 51 | 9 | 13 | 28 | 4 |
| 2 | 3 | 25 | 12 | 14 | 14 | 10 |
| 3 | 1.5 | 11 | 10 | 8 | 10 | 21 |
| 4 | 0.95 | 7 | 9 | 12 | 9 | 31 |
| 5 | 0.49 | 4.5 | 7 | 11 | 9.6 | 17 |
| Backup Filter | <0.49 | 2 | 54 | 42 | 30 | 16 |

## TABLE 4

### PREDICTION AND APPORTIONMENT OF LEAD AEROSOL

| | Mean Source Contributions (ng $m^{-3}$) | | | | Total Concentration (ng $m^{-3}$) | |
|---|---|---|---|---|---|---|
| Element | Soil | Automotive Emissions | Refuse | Refinery Emissions | Predicted | Observed |
| Al | 1060 | 0.9 | 27 | 5 | 1090 | 1100 ± 420 |
| As | 0.2 | 0 | 0.4 | 4.3 | 5.8 | 4.6 ± 6.7 |
| Br | 0.7 | 57 | 1.9 | 0.1 | 60 | 61 ± 28 |
| Na | 550 | 0 | 157 | 17 | 780 | 850 ± 540 |
| Pb | 4.2 | 163 | 144 | 264 | 575 | 1100 ± 1510 |
| Sb | 0.2 | 0 | 2.9 | 8.3 | 11.3 | 11.3 ± 17 |
| Zn | 4.2 | 1.3 | 230 | 4 | 240 | 638 ± 1040 |

appears to be the result of a combination of two sources, one composed of predominantly large particles and one of predominantly small particles. However, consideration of the possible sources of lead and of ratios between lead and other common marker elements in these sources, such as Al (soil), Br (automotive emissions) and As and Sb (lead alloying operations) /10/ indicates that four sources of airborne lead exist : large particle lead from soil, small particle lead from automobiles and refuse incineration and a combination large-and-small profile from the secondary lead refinery which was indicated by high correlation coefficients between Pb, As and Sb.

The chemical composition profiles for the sources considered are listed in Table 2 and the particle-size profiles are listed in Table 3. The actual model profiles were calculated by the combination of these two matrices. The soil size profile was obtained from the particle-size distribution of Sc since there are no major anthropogenic sources of this element. Aluminum size distributions were very similar indicating agreement and, by their lack of a large fine particle component, also indicating little evidence of coal combustion products. The elemental profile was obtained from analysis for 32 elements in over 50 soil and dust samples from throughout Metropolitan Toronto. /13/ The automotive elemental profile was derived from the literature /2/ and Pb/Br ratios from aerosol samples with little evidence of industrial contribution. The size profile for automotive emissions was determined from Pb and Br measurements on Toronto Island, aerosol samples from which show a strong Pb/Br correlation. It was assumed that, due to wind direction and sheer mass of the auto emission component, any confounding sources of Pb or Br were negligible.

TABLE 5

**MASS BALANCE L/S RATIOS**

| Element | 4 Sources Separate Sizes | 4 Sources Total Aerosol | 7 Sources Total Aerosol |
|---|---|---|---|
| Al | 1.8 | 1.01 | 1.33 |
| As | 1.9 | 1.26 | 1.19 |
| Br | 1.2 | 1.02 | 1.04 |
| Na | 1.4 | 1.09 | 1.02 |
| Pb | 1.7 | 1.9 | 2.0 |
| Sb | 1.6 | 1.0 | 1.02 |
| Zn | 2.2 | 2.7 | 1.2 |
| Average | 1.7 | 1.4 | 1.26 |

Pb/Al and Br/Al ratios for island soil /10/ were used to remove that component before profile calculation. The zinc in the automobile componemt, assumed to be from tire wear, was given a size profile similar to the soil profile, indicating erosion followed by dust entrainment rather than combustion. With this one exception, it was necessary to make the simplifying assumption that elemental ratios remained constant for all aerosol sizes from a given source; this was the result of a lack of available literature information.

The chemical profiles for refuse incineration and lead refining were obtained from a compilation of source profiles. /2/ The size profiles for lead refinery emissions were obtained from those of As since Pb, As and Sb were strongly correlated at the refinery site and because there were no other major sources of As indicated.

## Source Contributions

Table 4 lists the mean contributions of the four major sources of Pb aerosol to the ambient airborne particulate matter. These were obtained by least squares regression of the measured concentrations with the combination particle size and composition profile. Following a suggestion by Kowalczyk et al /12/,each elemental size fraction was weighted by $1/s^2$ where s is the analytical error in each concentration determination. This permits the CMB to weight all elements according to their analytical precision rather than according to the magnitude of their absolute concentrations.

The secondary lead refinery contributes the largest amount of airborne Pb, with a mean contribution of 46%. Airborne soil and dust particles contribute the least (<1%) with automotive emission and refuse incineration contributing approximately 25% and 28% respectively. The larger/smaller ratio in Table 4 is the larger of either the predicted-to-observed or observed-to-predicted ratio. The use of L/S ratios avoids the balancing of gross overpredictions and underpredictions and is thus a better measure of the balance fit. /6/ The L/S of 1.9 for lead is an indication either that the profiles for lead are slightly inaccurate or that other sources of Pb are contributing to the ambient aerosol. The particle-size profiles for Pb suggest no other source. It was therefore decided that the chemical profiles of the local lead sources were not identical to those listed in the literature. It is possible that variability in lead alloying operations at the secondary lead refinery was causing variability in the profile. This is supported by the excellent fit for Sb and the fact that lead was not included in the original fit.

Two measures of the accuracy of the balance are the number of negative source coefficients and the overall L/S ratio. The balance presented here produced no negative source coefficients and a L/S ratio of 1.4. This ratio is lower than for other CMB studies although the small number of elements fitted here precludes comparison with other work. On the other hand, most of the elements fitted here are elements for which other CMB models show relatively large L/S values. For example, Mizohata and Mamuro /14/ calculated the particle-size distribution of each source component in their overall balance by applying a CMB, using these same sources, to elemental data from each stage of a cascade impactor. They found large values of the observed-to-predicted ratio for both As (5.3) and Sb (3.2). These underpredictions were most evident in the fine particle fraction indicating additional sources of fine particulate not accounted for by the model. Scheff et al /4/, in their study of Chicago aerosol, also realized this problem in concluding that in their mass balance a greater proportion of respirable particulate remained unexplained (50%) than of total particulate (25%). Table 5 shows that, for the present model, there is not a large discrepancy between the L/S ratio for the overall balance on each element and the ratio for balances for each size fraction of each element, thus indicating that the source resolution accounted reasonably well for all fractions of the aerosol. This comparison constituted a third measure of the accuracy of the mass balance. A more accurate knowledge of elemental fractionation between particle sizes from different emission sources would further reduce the difference between the overall balance and the individual particle-size balances.

The L/S values in Table 4, indicate that zinc is the element fitted with the least accuracy. In a traditional CMB, in which there is no consideration of the size distribution of zinc, this underprediction would be accounted for by a larger Zn-rich refuse component as it is in the four-source model. However, consideration of the size distributions of Zn in Figure 2, as well as those of Pb, As and Sb, indicated that there was too much fine particle Zn to be accounted for by the refuse component. Given the existence of a nearby zinc-casting operation, two new size profiles were separated from the zinc particle-size distribution viz. a fine particle Zn vapour-condensation source (Z1) and a medium-sized zinc dust (Z2) source for which profiles are given in Tables 2 and 3. The chemical profile was obtained from zinc melting emissions. /2/ A salt profile was also considered since salt had been used as a road de-icer during three of the sampling periods, all of which had poorly predicted large particle Na concentrations.

Neither the salt nor the zinc profiles could be applied to all samples without causing many negative source coefficients, particularly with the zinc profiles since refuse incineration and the Zn vapour source are similar in Zn concentration and can be regarded as collinear in this data set. However, selective application, where size distributions indicated zinc or salt source contributions, yielded a balance with no negative source coefficients and, as shown in Table 5, a lower L/S ratio of 1.26; lower primarily as a result of the L/S for zinc being reduced from 2.7 to 1.2. The lower overall L/S indicates that including the two additional zinc sources in the model is consistent with the actual sources contributing to the ambient airborne particulate matter.

Increasing the number of elements available for fitting may improve the zinc condensate/refuse incineration separation but also will require consideration of all sources contributing to these additional elements. Work is now underway to examine size distributions for all of the elements determined and to consider other industrial sources of aerosol, such as cement production and steel processing, so that the particle-size CMB can be extended to resolve all of the sources of airborne particulate matter in the study area.

## CONCLUSIONS

This study presents a new receptor model, the size-specific elemental mass balance, which can be used to resolve the contributions of aerosol emission sources to different particle-size fractions of the ambient aerosol. Unlike the traditional CMB which utilizes only total elemental concentration, the SSEMB operates on differences in both the chemical composition and the particle-size distribution of emissions from the contributing sources, an approach which was found to be particularly useful where several of these contributing sources have chemically similar emissions, thus confounding the traditional "marker element" approach.

The potential of the new model was demonstrated by determining the contribution to the levels of Pb in the ambient aercsol of automotive exhaust, refuse incineration, soil and lead refinery emissions. The new model does not eliminate all elementally collinear sources but does reduce both the number of such sources and their effect on source apportionment. A more detailed analysis of elemental fractionation amongst particle sizes will be necessary to improve the accuracy of the model.

It was evident that a complimentary coupling of photonuclear and thermal neutron activation analysis, with the accompanying sensitivity, multi-elemental capability and instrumental nature of activation analysis, was essential to the development of the size-specific elemental mass balance.

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# Mutagenicity of Complex Mixtures of Polycyclic Aromatic Hydrocarbons in Ambient Air Particulate Matter

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## 1. Introduction

It has been estimated that 60% or more of the incidence of cancers in man and animals is due to the action of environmental chemicals or pollutants. Polycyclic Aromatic Hydrocarbons (PAH) are a particularly important group of carcinogenic pollutants in addition to many others that are formed through anthropogenic sources. PAH are not carcinogenic per se but may become so after metabolism to reactive products. PAH can be converted to a variety of metabolites. The microsomal mixed-function oxygenases have been implicated in the activation of PAH to toxic, mutagenic, and/or carcinogenic metabolites. These enzymes are multicomponent systems containing several forms of cytochrome P-450, a flavoprotein, NADPH-Cytochrome reductase, P-448 and other endogenous membrane-bound mono-oxygenase systems. Several mechanisms are involved in the alterations of monooxygenase activity by chemicals (Wiebel, 1980), such as the induction of a series of intracellular reactions that cause (1) an increase or decrease of enzymatic activity, (2) interference with catalytic processes, and (3) inactivation or destruction of the cytochrome.

Human exposure to PAH mixtures may produce carcinogenic effects depending on the ratios of the various strong carcinogens to weak- or non-carcinogenic constituents.

## 2. Materials and Methods

Standard Reference material (SRM) 1649 was purchased from U.S. National Bureau of Standards. SRM 1649 is a mixture of air particulate matter available in the form of dust. Individual PAH have been purchased from Standard Chemical companies.

### Mice

Virgin B6C3F1 female mice, 8-9 weeks of age, were used for the *in vivo* bone marrow micronucleus experiments. The mice ranged from 20-22 g in weight and they were fed with Purina Laboratory Rodent Chow and watered ad libitum. The B6C3F1 mouse strain is being used because the mono-oxygenase enzyme system in this strain can be induced by polycyclic aromatic hydrocarbons (Nerbert et al., 1972; Green, 1973).

### 2.1 Methods

The *in vivo* bone marrow micronucleus assay (Heddle and Salamone, 1981) has been used to test the ambient air particulate matter.

### In Vivo Bone Marrow micronucelus Assay

In general, people are exposed to air and particulate matter that could be mutagenic and thus hazardous and a matter of concern. Chromosomal changes comprise a major part of this genetic danger.

The traditional method of examining mitotic chromosomes at metaphase for analysis is tedious and time consuming. Since humans cannot be used in a direct test, a mammalian in vivo test system is very desirable. Therefore, for test purposes bone marrow of common laboratory rodents are an appropriate choice. As an alternative to metaphase scoring, measurement of the micronuclei is appropriate and desirable. Micronuclei are formed in the cytoplasm from fragments of chromosomes left behind at anaphase (because they lack centromeres). Micronuclei persist for some time and so may be scored at interphase. Since most of the cells of a tissue are in interphase there is virtually no time spent finding cells to score (Heddle, 1973).

## 3. Experimental Results

In the urban air particulate matter of SRM 1649, the following PAH have been identified:

1. Phenanthrene
2. Fluoranthene
3. Pyrene
4. Chrysene
5. Benz(a)anthracene
6. Perylene
7. Benzo(b)fluoranthene
8. Benzo(k)fluoranthene
9. Benzo(a)pyrene
10. Benzo(e)pyrene
11. Benzo(ghi)perylene
12. Dibenz(a,h)anthracene
13. Indeno(1,2,3-cd)pyrene

Carcinogenic and mutagenic potencies of the above PAH have been reported earlier. From the above list they can be broadly classified as:

(i) non-carcinogenic and non-mutagenic (compounds 1, 3)

(ii) slightly carcinogenic and mutagenic (compounds 4, 5, 6)

(iii) more carcinogenic and mutagenic (compounds 7, 9, 12). Based on the above data, and also depending on the structural similarities, we tried to select complex mixtures of mutagenic-mutagenic, mutagenic-non mutagenic and non mutagenic-non mutagenic substances.

### 3.1 Lethal Dose$_{50}$

A group of 5 mice (both male and female) were treated in each treatment to test the $LD_{50/7}$ of the SRM 1649. The doses of SRM 1649 ranged from 100 mg/kg to 4000 mg/kg.

Air particulate matter of SRM 1649 (in the form of dust) was dissolved in DMSO in appropriate amounts. The mixture was shaken thoroughly in a vortex and the suspension allowed to settle. The supernatant was drawn off and transferred to a separate amber vial. Different dilutions were made and the mice were injected i.p. The mice were checked daily for 7 days for sickness or deaths. The mice used were $B6C3F_1$ females and males. $LD_{50}$ was calculated according to Weil (1952). $LD_{50/7}$ of SRM 1649 was found to be 535 mg/kg.

### 3.2 Dose Response Studies with SRM 1649

SRM 1649, in the form of dust, was weighed, dissolved in DMSO and allowed to settle. The supernatant was taken and the appropriate dilutions were made.

The doses were adjusted to 250 mg/kg, 500 mg/kg and 1000 mg/kg per body weight of mouse. The mice were injected i.p. with SRM 1649 supernatant. Five mice were used per treatment and samples were collected at 24, 48 and 72 hrs after treatment for testing with the _in vivo_ bone marrow micronucleus assay.

The results indicated that there was no dose-related response of the micronucleated polychromatic erythrocytes (MNPCE). In the 250 mg/kg treatment, on an average, 1.5 MNPCE/1000 PCE at 24 hr, 1.8 MNPCE/1000 PCE at 48 hr, and 1.0 MNPCE/1000 PCE at 72 hr were observed. In the 500 mg/kg treatment, on an average, 1.0 MNPCE/1000 PCE, 1.8 MNPCE/1000 PCE, and 1.0 MNPCE/1000 PCE at 24, 48 and 72 hr respectively were observed. In the 1000 mg/kg treatment, it was observed that 2.8 MNPCE/1000 PCE at 24 hr, 1.6 MNPCE/1000 PCE at 48 hr and 1.0 MNPCE/1000 PCE at 72 hr respectively.

## Dose Response Studies with the Reconstituted Mixture of the PAH Present in the Ambient Air Particulate Matter (SRM 1649)

**Table 1. Details of the reconstituted mixture of SRM 1649**

| PAH | Available in SRM 1649 mg/kg | 5000 X of the doses available in SRM 1649, adjusted to the weight of mouse and in 0.1 ml DMSO. (g/kg |
|---|---|---|
| 1. Phenanthrene | 4.8±0.3 | 0.50 |
| 2. Fluoranthene | 7.4±0.8 | 0.80 |
| 3. Pyrene | 6.3±0.4 | 0.65 |
| 4. Chrysene | 3.5±0.1 | 0.35 |
| *5. Benz(a)anthracene | 2.5±0.3 | 0.25 |
| 6. Perylene | 0.8±0.04 | 0.08 |
| *7. Benzo(b)fluoranthene | 6.2±0.4 | 0.65 |
| 8. Benzo(k)fluoranthene | 2.0±0.2 | 0.20 |
| 9. Benzo(a)pyrene | 2.6±0.4 | 0.30 |
| 10. Benzo(e)pyrene | ---- | --- |
| 11. Benzo(ghi)perylene | 3.9±0.8 | 0.45 |
| 12. Dibenz(a,h)anthracene | 0.54±0.04 | 0.05 |
| *13. Indeno(1,2,3-cd)pyrene | 3.4±0.4 | 0.35 |

* However, at the time of experiments Benz(a)anthracene, Benzo-(b)fluoranthene and Indeno(1,2,3-cd)pyrene could not be included since these PAH were not readily available.

From the stock solution A (5000 X) dilutions B (2500 X) and C (1250 X), were made.

So, the dose response studies were conducted with 1250 X, 2500 X and 5000 X of the mixture of PAH available in the SRM 1649. Following the intra-peritoneal injections, the bone marrow samples were collected to score the micronucleated polychromatic erythrocytes at various sampling times.

The results are tabulated hereunder:

**Table 2. Frequency of MNPCE obtained with different doses of reconstituted mixture of SRM 1649**

| DOSE | * Average no. MNPCE/1000 PCE + S E M<br>Sampling Time (hr) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| A. 5000 X | 2.8±0.7 | 5.3±1.0 | 2.3±0.9 |
| B. 2500 X | 1.0±0.6 | 5.0±1.7 | 4.3±1.0 |
| C. 1250 X | 2.0±0.9 | 11.0±3.1 | 4.3±1.6 |

* 5 mice/treatment were used.

From the above results, it is clear that the maximum occurrence of MNPCE (11.0±3.1) were observed at 48h sampling time with the lowest dose of 1250 X.

### 3.3 Dose Response of Mixture of Mutagens

BaP and DMBA have been chosen to study the dose response in the form of a mixture. Both these mutagens are promutagens. The $LD_{50/7}$ of BaP and DMBA are 232 mg/kg and 84 mg/kg respectively in the mice. Three doses have been chosen to study the dose response of individual mutagens, whereas BaP was kept constant and the doses of DMBA were varied in the mixture. In this study, 1/12, 1/6 and 1/2 of $LD_{50}$ doses have been chosen. Thus, 21 mg/kg, 42 mg/kg and 125 mg/kg for BaP, and 7 mg/kg, 14 mg/kg and 42 mg/kg for DMBA were used. In the case of the mixture 21 mg/kg of BaP was kept constant and the dose of DMBA was varied using 7 mg/kg, 14 mg/kg and 42 mg/kg. From our earlier experience, we know that the peak occurrence of micronucleated polychromatic erythrocytes was at 48 h after the administration of mutagen to the mice. So, in the present experiments the bone marrow samples were collected only at 48 h. The results are tabulated in Tables 3 and 4.

**Table 3: Frequency of MNPCE/1000 PCE from bone marrow samples collected at 48 h after the administration of mutagens to mice.**

| Mutagen | Of $LD_{50}$ | Dose (mg/kg) | MNPCE/1000 PCE ± SEM |
|---|---|---|---|
| BaP | 1/12 | 21 | 2.0 ± 0.6 |
| | 1/6 | 42 | 2.4 ± 0.5 |
| | 1/2 | 125 | 5.2 ± 1.2 |
| DMBA | 1/12 | 7 | 7.0 ± 1.0 |
| | 1/6 | 14 | 6.0 ± 0.6 |
| | 1/2 | 42 | 12.8 ± 2.2 |
| BaP + DMBA | 1/12 + 1/12 | 21+7 | 1.4 ± 0.4 |
| | 1/12 + 1/6 | 21+14 | 2.4 ± 0.7 |
| | 1/12 + 1/2 | 21+42 | 4.4 ± 0.9 |

**Table 4:** **Data showing the inhibitory effect of promutagens at varying doses.**

| Mutagens | Dose (mg/kg) | MNPCE/1000PCE (If Additive) | MNPCE/1000PCE (Actual) | Difference | % Inhibition |
|---|---|---|---|---|---|
| BaP + DMBA | 21+7 | 2.0+7.0 = 9.0 | 1.4 | 7.6 | 84.4 |
| | 21+14 | 2.0+6.0 = 8.4 | 2.4 | 6.0 | 71.4 |
| | 21+42 | 2.0+12.8=14.8 | 4.4 | 10.4 | 70.3 |

Similarly, in another set of experiments dose response was studied with 1/4, 1/5 and 1/10 of $LD_{50}$ of BaP, 1/2, 1/4, 1/5 and 1/10 $LD_{50}$ of DMBA. In the mixture BaP was kept constant with 1/10 $LD_{50}$ and the doses of DMBA varied. The results are presented in Tables 5 and 6, which clearly indicate that inhibition occurs when these promutagens are together in the system (Raj and Katz, 1983). Similar results have been reported earlier from our laboratory (Katz and Raj, 1983; Raj and Katz, 1985).

**Table 5:** **Frequency of MNPCE/1000 PCE from bone marrow samples collected at 48 h after the administration of the mutagens in mice.**

| Mutagen | of $LD_{50}$ | Dose (mg/kg) | MNPCE/1000PCE ± SEM |
|---|---|---|---|
| BaP | 1/10 | 25.0 | 2.6 ± 0.8 |
| | 1/5 | 50.0 | 4.6 ± 0.08 |
| | 1/4 | 62.5 | 4.0 ± 1.1 |
| DMBA | 1/10 | 8.4 | 4.2 ± 0.7 |
| | 1/5 | 16.8 | 4.6 ± 0.7 |
| | 1/4 | 21.0 | 4.2 ± 0.8 |
| | 1/2 | 42.0 | 12.8 ± 2.2 |
| BaP + DMBA | 1/10+1/10 | 25.0+8.4 | 2.6 ± 0.9 |
| | 1/10+1/5 | 25.0+16.8 | 3.8 ± 0.7 |
| | 1/10+1/4 | 25.0+21.0 | 5.0 ± 1.4 |
| | 1/10+1/2 | 25.0+42.0 | 5.0 ± 1.4 |

**Table 6:** Data showing the inhibitory effect of promutagens at varying doses.

| Mutagens | Dose (mg/kg) | MNPCE/1000PCE (If Additive) | MNPCE/1000PCE Actual | Difference | % Inhibition |
|---|---|---|---|---|---|
| BaP+DMBA | 25+8.4 | 2.6+4.2 =6.8 | 2.6 | 4.2 | 62 |
| | 25+16.8 | 2.6+4.6 =7.2 | 3.8 | 3.4 | 47 |
| | 25+21.0 | 2.6+4.2 =6.8 | 5.0 | 1.8 | 26 |
| | 25+42.0 | 2.6+12.8=15.4 | 5.0 | 10.4 | 68 |

### 3.4 Dose Response Studies of Benzo(ghi)perylene in the in vivo Bone Marrow Micronucleus Assay

The LD$_{50}$ of Benzo(ghi)perylene is more than 500 mg/kg in mice. The doses 1/8 of LD$_{50}$ (62.5 mg/kg), 1/4 LD$_{50}$ (125 mg/kg) and 1/2 LD$_{50}$ (250 mg/kg) of Benzo(ghi)perylene were selected in this study. The chemical was dissolved in DMSO and the mice were injected intraperitoneally. Groups of five mice per treatment were used. Bone marrow samples were collected at 24, 48 and 72 hrs after the administration of the chemical. In all the treatments the average number of micronucleated polychromatic erythrocytes per 1000 PCE were less than two which is equivalent to the control background. No dose-related response was observed.

### 3.5 Dose Response Studies with Benzo(k)fluoranthene

B6C3F1, females, 8 weeks old, were injected i.p. with either 25 mg/kg, 50 mg/kg or 100 mg/kg of Benzo(k)fluoranthene that was dissolved in DMSO. A group of 5 mice were used per each treatment. The bone marrow samples were collected at 24, 48, and 72 hrs after the administration of the chemical. The number of micronucleated polychromatic erythrocytes were scored per 1000 PCE. The results indicated that there was no dose-related response between the amount of mutagen injected and the MNPCE. A maximum number of 3 MNPCE/1000 PCE was observed at 48 hr sampling period in 50 mg/kg treatment.

## 3.6 **Studies on the Effect of Mixtures of PAH, with the in vivo Bone Marrow Micronucleus Assay**

The mutagens that were selected for this study included DMBA, BaP, and Benzo(ghi)perylene and their doses were 21 mg/kg, 58 mg/kg and 150 mg/kg respectively. Individual mutagens, a mixture of pairs of mutagens, (DMBA + BaP; DMBA + Benzo(ghi)perylene or BaP + Benzo(ghi)perylene), or a mixture of all the three mutagens were used. A group of 5 mice per treatment were included. Bone marrow samples were collected at 24, 48 and 72 hours after the administration of the mutagens. Micronucleated polychromatic erythrocytes /1000 PCE were scored. The results indicated that there is no spectacular additive effect even when all the three mutagens are included. But, as expected, there was about 65% inhibition in the occurrence of MNPCE from the mice that received a complex mixture of DMBA and BaP. The results are presented in the following Table 7.

**Table 7:** **Frequency of MNPCE/1000 PCE in the mice that received different mutagens either individually or in mixture**

| Mutagens | MNPCE/1000 PCE ± SEM | | | |
|---|---|---|---|---|
| | 24h | 48h | 72h | Comments |
| DMBA | 1.6±0.5 | 8.4±1.4 | 2.2±0.5 | |
| BaP | 2.0±0.8 | 2.4±0.5 | 1.2±0.4 | |
| Benzo(ghi)perylene | 0.4±0.3 | 0.8±0.4 | 2.4±1.0 | |
| DMBA+BaP | 2.0±0.8 | 3.0±0.6 | 3.2±0.5 | About 65% inhibition at 48h compared to DMBA |
| DMBA+Benzo(ghi)perylene | 2.6±0.9 | 2.4±0.8 | 2.0±0.7 | |
| BaP+Benzo(ghi)perylene | 2.2±0.8 | 2.4±0.3 | 1.2±0.7 | |
| DMBA+BaP+Benzo(ghi)perylene | 0.75±0.22 | 2.0±0.9 | 3.8±0.09 | No additive effec |

## 4. Conclusions:

1. LD$_{50/7}$ of standard reference material 1749 was found to be 535 mg/kg when tested in mice.
2. No dose related response was observed either with SRM 1649, or Benzo (k) fluoranthene, or Benzo (ghi) perylene in the *in vivo* bone marrow micronucleus assay.
3. No additive response was observed in the *in vivo* bone marrow nucleus assay when the PAH were tested as complex mixtures, e.g., BaP+DMBA; BaP+DMBA + Benzo (ghi) perylene; BaP + Benzo (ghi) perylene; or DMBA + Benzo (ghi) perylene.

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# DOSE-RESPONSE STUDIES OF GASEOUS POLLUTANTS ON FOOD CROPS

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## ABSTRACT

A long-term dose-response study was conducted in a controlled environment facility using 'Spartan Bonus' carrot plants. Fifteen-day old plants were exposed to either 0, 0.06, 0.12, or 0.18 ul $l^{-1}$ $O_3$ for seven hours one day each week for a total of 5 weeks. Shoot and root growth analyses were conducted on plants which were harvested one week after each exposure. Leaf area and leaf fresh and dry weights were significantly decreased by the 0.18 treatment after one exposure. Leaf injury was significantly increased by the 0.12 and 0.18 treatments after one exposure. Root fresh and dry weights were significantly decreased in the 0.12 and 0.18 treatments after the third exposure. By the fifth week, significant reductions in leaf and root parameters occurred in the 0.12, as well as the 0.18 treatments. No significant changes were detected in dry matter partitioning between the roots and shoots in any of the treatments.

An experiment was performed, using 14-day old 'New Yorker' tomato plants, to determine changes in plant growth and physiology following exposure to $O_3$ levels (0, 0.035, 0.070, and 0.105 ul $l^{-1}$) below those that normally produce visible leaf injury. Leaf and stem growth parameters, stomatal conductance, and chlorophylls a and b were analyzed three days following the seven hour exposure. Neither growth nor chlorophyll was significantly affected by the treatments. Three days after the exposure, stomatal conductance was significantly reduced in all the $O_3$ treatments.

Research was conducted to determine if comparisons could be made between indoor and outdoor $O_3$ responses of several crop species. Seeds for indoor experiments of four soybean, two corn, and three wheat cultivars were obtained from the USEPA NCLAN program. These same cultivars had been grown and subjected to $O_3$ treatments in open-top field chambers in NCLAN studies. In the present experiment, the seedlings were exposed to 0, 0.06, 0.12, or 0.24 ul $l^{-1}$ $O_3$ for one seven hour period in controlled environment facilities. The results suggested that a good comparison could not be made between indoor and outdoor experiments determining the effect of $O_3$ using these species.

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A long-term dose-response study was conducted in a controlled environment facility using 'Spartan Bonus' carrot plants. Fifteen-day old plants were exposed to either 0, 0.06, 0.12, or 0.18 $\mu l\ l^{-1}\ O_3$ for seven hours one day each week for a total of 5 weeks. Shoot and root growth analyses were conducted on plants which were harvested one week after each exposure. Leaf area and leaf fresh and dry weights were significantly decreased by the 0.18 treatment after one exposure. Leaf injury was significantly increased by the 0.12 and 0.18 treatments after one exposure. Root fresh and dry weights were significantly decreased in the 0.12 and 0.18 treatments after the third exposure. By the fifth week, significant reductions in leaf and root parameters occurred in the 0.12, as well as the 0.18 treatments. No significant changes were detected in dry matter partitioning between the roots and shoots in any of the treatments.

An experiment was performed, using 14-day old 'New Yorker' tomato plants, to determine changes in plant growth and physiology following exposure to $O_3$ levels (0, 0.035, 0.070, and 0.105 $\mu l\ l^{-1}$) below those that normally produce visible leaf injury. Leaf and stem growth parameters, stomatal conductance, and chlorophylls a and b were analyzed three days following the seven hour exposure. Neither growth nor chlorophyll was significantly affected by the treatments. Three days after the exposure, stomatal conductance was significantly reduced in all the $O_3$ treatments.

Research was conducted to determine if comparisons could be made between indoor and outdoor $O_3$ responses of several crop species. Seeds for indoor experiments of four soybean, two corn, and three wheat cultivars were obtained from the USEPA NCLAN program. These same cultivars had been grown and subjected to $O_3$ treatments in open-top field chambers in NCLAN studies. In the present experiment, the seedlings were exposed to 0, 0.06, 0.12, or 0.24 $\mu l\ l^{-1}$ $O_3$ for one seven hour period in controlled environment facilities. The results suggested that a good comparison could not be made between indoor and outdoor experiments determining the effect of $O_3$ using these species.

## INTRODUCTION

Ozone is a common air pollutant present at various times and levels in the commercial crop producing areas of southern Ontario. Past research has indicated that $O_3$ sensitivity of plants is species as well as cultivar dependent. Some agricultural crops have had more attention than others concerning $O_3$ sensitivity of growth and yield.

Ozone episodes occur throughout a growing season. Bennett and Oshima (1976) indicated that intermittent six hour exposures of carrot plants to $O_3$ at 0.19 and 0.25 $\mu l\ l^{-1}$ over a growing season significantly reduced total fresh weight as well as root dry weight at the end of the season. Some of our past studies indicated that leaf growth responses of 16 and 21 day old carrot plants were different when exposed to 0.18 $\mu l\ l^{-1}$ $O_3$ for one seven hour period (Ormrod and

Petitte, 1985). No data has been reported to show the effects of intermittent low $O_3$ levels over a growing season. The objective of this experiment was to determine the effects of weekly exposures of carrot plants to the same $O_3$ levels over a period of five weeks.

Air pollution injury can be classified as either hidden or visible (Heath, 1980). Hidden injury has been defined as biochemical or physiological alterations resulting in decreased plant productivity without visible injury (Heath, 1980). In previous studies (unpublished) using 16-day old tomato seedlings, the results suggested that no significant visible injury occurred on the plants below 0.12 $\mu l\ l^{-1}\ O_3$. However, specific leaf area was significantly reduced at 0.12 $\mu l\ l^{-1}\ O_3$, indicating that the photosynthetic leaf surface area was decreasing faster than leaf dry weight. An experiment was conducted to study hidden injury (growth, stomatal conductance, chlorophyll concentrations) in tomato plants exposed to low levels of $O_3$.

Experiments to quantify the effects of $O_3$ on crop growth are needed to permit evaluations of the differences in sensitivity among species, as well as to provide dose-response relationships for the predictions of field effects of $O_3$ episodes. Previous controlled environment indoor studies and field studies in open-top chambers (Petitte and Ormrod, 1986) have indicated that the sensitivity of a plant to $O_3$ in the indoor compared to the outdoor environment depends upon species, age, and stresses on the plant. Over the past several years, the United States Environmental Protection Agency's National Crop Loss Assessment Network (NCLAN)(Heck et al., 1984) has conducted field experiments in which cultivars of specific species are exposed to $O_3$ throughout the growing season. No experiments to test the sensitivity of these same cultivars to indoor $O_3$ exposures has been reported. The objective of our experiment was to determine if the sensitivity of the young plants would have any relationship to the results obtained in the outdoor experiments.

## MATERIALS AND METHODS

### Experiment 1

'Spartan Bonus' carrot seeds were planted in Promix BX (1:1:3 mixture of perlite:vermiculite:sphagnum peat moss) soilless media in white plastic cylindrical containers (200 mm high x 80 mm wide) with five 8 mm holes drilled into the bottom of each to allow for excess water runoff. A one-half strength complete nutrient solution (Hoagland and Arnon, 1950) was supplied daily to the plants. The plants were grown in Conviron Model E8 growth chambers at standard baseline growth conditions (Ormrod et al., 1980). Fourteen days after seeding, 21 plants were placed into each of four continuous stirred tank reactor (CSTR) chambers (Heck et al., 1978; Le Sueur-Brymer and Ormrod, 1984) to allow for approximately an 18 hour acclimation period prior to treatment. The environmental conditions in the CSTR chambers were similar to those described for the growth chamber.

The following day immediately prior to treatment, planar leaf area (PLA) (Ormrod et al., 1983) was measured as a covariate on each plant. One plant was then harvested from each chamber for a 0 day growth point. One of the following $O_3$ treatments was then imposed upon the plants in one of the four CSTR chambers: 0, 0.06, 0.12, 0.18 $\mu l\ l^{-1}$. Ozone was produced and monitored by a Grace high-voltage generator and a Dasibi (Model 1003-AH) analyzer, respectively. The exposure period continued for seven hours. Following treatment, the plants were placed back into the growth chamber for seven days and then put back into the CSTR chambers. The following day, all plants were rated for leaf injury based upon the percent of visible injury. Four plants from each treatment were then harvested and measurements of leaf area and leaf fresh and dry weights were conducted. Planar leaf area was again measured on the remaining 16 plants in each treatment. Each plant was then exposed for seven hours to the same treatment it had received the previous week. Following exposure, the plants were placed back into the growth chamber for seven days. The same procedure described for the first exposure was continued for a total of five weeks of exposure. Each time all the plants were rated for injury and four plants from each treatment were harvested seven days following the $O_3$ treatment. From the second exposure onward, both leaf and root measurements were conducted.

The experimental design was a randomized complete block with each block a complete repetition of the experiment. All treatments were replicated three times except for 0.18 $\mu l\ l^{-1}$ which consisted of two replications following the second exposure period. Each treatment in each block was composed of four subsamples. The subsample mean of each block was used in the data analyses. All growth variables were transformed to natural logarithms prior to further analysis. This step was performed in order to normalize the responses and stabilize their variances. Transformation of foliar injury was not required.

The results of the experiment were analyzed using a 3-dimensional response surface model for each growth variable. The covariate, PLA, was used for leaf area and leaf and root fresh and dry weights.

Each response variable was fitted to a second order mutliple regression response surface. The form of the linear model for each response surface used was:

$$Y_i = B_i + \alpha_1(\text{covariate}) + \alpha_2(\text{AGE}) + \alpha_3(O_3) + \alpha_4(\text{AGE})^2 + \alpha_5(O_3)^2 + \alpha_6(\text{AGE} \times O_3)$$

where B = blocks.

After the surface was fitted, the interaction coefficient was tested using an F test with a significance level of 0.10. If the AGE x $O_3$ interaction was not significant, the effects of AGE and $O_3$ alone were tested by dropping terms out of the model. Contour plots were made of the final models.

**Experiment 2**

'New Yorker' tomato seeds were planted and cultured in a Conviron Model E8 growth chamber as reported in Ormrod and Petitte (1985). On the 14th day of growth, covariate measurements of plant height, PLA (Ormrod et al., 1983), and plastochron index were made on each plant prior to placement in the CSTR chambers. The environment of the CSTR chambers was similar to that in the growth chamber. One of the following $O_3$ treatments was then imposed upon the plants in one of the four CSTR chambers for seven hours: 0, 0.035, 0.070, and 0.105 $\mu l\ l^{-1}$. Ozone was generated and monitored as described in Experiment 1. Following treatment, the plants were placed back into the growth chamber. Each morning thereafter about three hours into the photoperiod, stomatal diffusive resistance was measured on the abaxial leaf surface of the first leaf of each plant with a LI-COR 700 transient porometer. On the third day following the exposure, leaf discs of known area were cut from two plants of each treatment, frozen in 80% (w/v) acetone, and kept in the dark for later analysis of chlorophylls a and b (Strain and Svec, 1966). An equal area of leaf discs from the same leaves were dried to base chlorophyll content on leaf dry weight. Post harvest growth measurements included leaf area and leaf and stem fresh and dry weights.

The experimental design was a randomized complete block replicated over time with three blocks. In each treatment, three subsamples were used to determine growth parameters and two subsamples were used in chlorophyll analysis. Stomatal resistance was measured on all the subsamples and converted to stomatal conductance for analysis. The growth variables were transformed to their natural logarithms for analysis of covariance using PLA as the covariate. The mean of the subsamples of each treatment was used in the data analysis.

### Experiment 3

#### Soybeans

Seeds of four soybean cultivars ('Williams-79', 'Amsoy-71', 'Corsoy-79', 'Pella') used in the NCLAN experiments were obtained from Argonne National Laboratory and were planted and cultured in a Conviron Model E8 growth chamber as described by Ormrod and Petitte (1985). Twelve days later the plants were placed into the CSTR chambers for approximately 24 h prior to the $O_3$ exposure. This allowed for acclimation of the plants to CSTR conditions even though they were similar to those of the growth chamber. Following the acclimation period, plastochron index was measured as a covariate on each plant. One of the following $O_3$ treatments was then imposed upon the plants in one of the four CSTR chambers for seven hours: 0, 0.035, 0.070, 0.105 $\mu l\ l^{-1}$. Ozone was generated and monitored as described in Experiment 1. The plants were returned to the growth chamber following exposure. Visible injury was determined as described in Experiment 1 three days following exposure immediately prior to the harvest. Leaf area and leaf and stem fresh and dry weights were measured at harvest.

The experimental design was a randomized complete block completely replicated three times over time with four subsamples per treatment per block for 'Corsoy-79' and 'Amsoy-71', and two subsamples per treatment per block for 'Pella' and 'Williams-79'. The means of the subsamples for each treatment of each block were transformed to their natural logarithms, and plastochron index was used as the covariate in the analysis of covariance.

### Corn and Wheat

Seeds of two corn ('Pioneer 3780', 'PAG 397') and three wheat ('Roland', 'Abe', 'Arthur') cultivars used in the NCLAN experiments were obtained for Argonne National Laboratory, planted and cultured as described for the soybeans. Ozone exposure was exactly as described for the soybeans, except that the plants were 11 days old. Plant height was measured prior to exposure and used as the covariate in the final analyses. Injury rating and harvesting occurred three days following the exposure. Leaf area and fresh and dry weights were measured for each species. The experimental design and the analysis of covariance were as described for the soybeans. Each treatment of each block consisted of four subsamples.

## RESULTS AND DISCUSSION

## Experiment 1

Leaf injury in the 0.12 and 0.18 $\mu l\ l^{-1}$ treatments was significantly higher than that in the control carrot plants one week following the seven hour exposure to $O_3$ (TABLE 1). This observation was made throughout the remainder of the experiment. Injury was not cumulative over the five week period of exposures and appeared to peak and slightly decline after the fourth weekly exposure. After the third exposure, plants in the 0.06 $\mu l\ l^{-1}$ $O_3$ treatment had some injury, but were not significantly different from the control plants.

Growth was significantly reduced in some of the 0.12 and 0.18 $\mu l\ l^{-1}$ treatments during the experiment (TABLE 1). One week following the first seven hour exposure, the 0.18 $\mu l\ l^{-1}$ $O_3$ treated plants had significant reductions in leaf area as well as leaf fresh and dry weights. Leaf area was also significantly reduced in the 0.12 $\mu l\ l^{-1}$ treatments.

Following the second exposure, leaf fresh and dry weights were significantly reduced in the two highest $O_3$ treatments (TABLE 1). No effects were observed in roots or leaf/root dry weight ratio.

Leaf area and leaf fresh and dry weights significantly decreased in the 0.18 $\mu l\ l^{-1}$ treatment following the third weekly exposure (TABLE 1). Root fresh and dry weights were reduced in the 0.12 and 0.18 $\mu l\ l^{-1}$ $O_3$ treatments.

Even though reductions in growth parameters were observed following the fourth exposure, variation in the data was too great to obtain differences at the $P<0.10$ level of significance (TABLE 1).

Following the fifth week of exposure, significant reductions were observed in all the leaf and root parameters at the 0.12 and 0.18 $\mu l$

$l^{-1}$ $O_3$ treatments (TABLE 1).

No interactive effect of $O_3$ concentration with the age of the carrot plant at the time of exposure was observed on leaf to root dry weight ratio (TABLE 2). The nonuniform spacing of the contour lines indicated that the response of the partitioning of the dry matter between the leaves and roots decreased as the plant aged (FIGURE 1). The X axis refers to age of the plant at time of exposure to $O_3$. Plants exposed at 15, 22, 29, 36, or 43 days had 1, 2, 3, 4, or 5 exposures, respectively. The spacing of the contours widened with increasing age because the quadratic component was significant.

Root fresh and dry weights each displayed a complex antagonistic interaction pattern influenced by linear and quadratic responses and a negative significant interaction of age at exposure and $O_3$ concentration (TABLE 2). If hypothetical lines are drawn across the graph of root fresh weight perpendicular to the Y axis at 0.06, 0.12 and 0.18 $\mu l$ $l^{-1}$ interpretation of the effects will be facilitated (FIGURE 2). At 0 $\mu l$ $l^{-1}$ $O_3$ concentration root fresh weight was at its maximum during the exposure period. A seven hour dose of $O_3$ at 0.06 $\mu l$ $l^{-1}$ each week for four weeks did not reduce fresh weight significantly. However, the fifth exposure caused some reduction. At 0.12 $\mu l$ $l^{-1}$ $O_3$, the contour lines begin to curve on about day 36. This indicates that in order for the plants at this concentration of $O_3$ to have the same root weight as the control plants (0 $\mu l$ $l^{-1}$), they would have to be about 39 days old. The fifth exposure to $O_3$ at 0.12 $\mu l$ $l^{-1}$ significantly reduced root fresh weight when compared to the control plants.

A decrease in root fresh weight was observed in those plants exposed to 0.18 $\mu l\ l^{-1}$ $O_3$ after the first week of exposure. This reduction due to $O_3$ and the age of the plant as well as their interaction continued throughout the growing season.

Quadratic dose-response functions represented the effects of age at exposure and $O_3$ concentration on leaf area (FIGURE 3) and fresh and dry weights (TABLE 2). All the response variables showed significant additive decreases (linear and quadratic effects) from age and $O_3$ effects. Leaf growth decreased faster in the plants receiving $O_3$ throughout the season than in those plants receiving no $O_3$.

The use of response surfaces and contour plots allows one to explain the interaction of $O_3$ concentration with the age of the plant at exposure. Because $O_3$ episodes are prevalent throughout a growing season for most crops in populated areas, these results indicate the detrimental interaction of stage of growth with $O_3$ concentration on carrot root production.

**Experiment 2**

Stomatal conductances of tomato for the first, second, and third days following exposure to $O_3$ are recorded in TABLE 3. Conductance was not significantly affected by the $O_3$ until the third day following exposure when all the $O_3$ treatments had significant reductions in comparison to the control. Beckerson and Hofstra (1979) reported decreasing stomatal conductances in soybean, cucumber, and radish leaves over five consecutive days (6 h $d^{-1}$) of exposure to 0.15 $\mu l\ l^{-1}$ $O_3$. Visible injury did not appear until the last half of the second day of the exposure. The response of stomata on the tomato leaves to a previous $O_3$ exposure, as observed in the present experiment, indicates the extreme sensitivity of the stomata to low levels of the pollutant.

Neither growth nor chlorophyll a or b was significantly affected by the $O_3$ treatments. Future studies should allow the plants to grow for several more days following the exposure to determine if stomatal conductances of the $O_3$ ozone treated plants would recover to the levels of the control plants. Visible injury was not present in any of the treatments.

**Experiment 3**

**-----Soybean**

Neither 'Corsoy-79', 'Pella', nor 'Williams-79' soybean plants was significantly affected by the $O_3$ treatments. 'Amsoy-71', however, did have significant reductions in leaf area and leaf fresh and dry weights at 0.24 $\mu l\ l^{-1}$ $O_3$ (TABLE 4). Heck et al. (1984) reported the predicted relative yield losses at a seasonal 7 h $d^{-1}$ mean $O_3$ concentration of 0.09 $\mu l\ l^{-1}$ for 'Corsoy' and two 'Williams' cultivars to be 34.8, 29.1 and 25.9%, respectively. No data were immediately available for either 'Pella' or 'Amsoy' cultivars.

**-----Corn**

No significant differences were observed between the treated and the control plants. 'PAG 397' and 'Pioneer 3780' cultivars had predicted relative yield losses of 8.1 and 16.7%, respectively, at a seasonal 7 h $d^{-1}$ mean $O_3$ concentration of 0.09 $\mu l\ l^{-1}$ (Heck et al., 1984).

**-----Wheat**

Leaf dry weight of 'Abe' wheat plants increased significantly at 0.06 and 0.24 $\mu l\ l^{-1}$ $O_3$ (TABLE 5), while the leaf dry weight of 'Roland' plants decreased significantly. 'Arthur' wheat plants were unaffected by the $O_3$. 'Abe', 'Arthur', and 'Roland' cultivars had predicted relative yield losses of 26.7, 27.4, and 45.4%, respectively, at a seasonal 7 h $d^{-1}$ mean $O_3$ concentration of 0.09 $\mu l$ $l^{-1}$ (Heck et al., 1984).

### -----Summary

The majority of these cultivars did not behave indoors under the stress of one seven hour $O_3$ exposure as they did outdoors under a seasonal exposure to $O_3$. The effects of $O_3$ on early growth was not a good indicator of the effect on final yield. Perhaps an indoor exposure to $O_3$ may be used as an indication of yield effects in the field if the plants are exposed at a more sensitive stage of development.

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TABLE 1. Geometric means for response variables. Each response has been adjusted to a common initial size (analysis of covariance).

| Response variable | Age of plant at exposure (days) | Ozone concentration ($\mu l\ l^{-1}$) | | | |
|---|---|---|---|---|---|
| | | 0.00 | 0.06 | 0.12 | 0.18 |
| Leaf area ($cm^2$) | 15 | 28.30 | 28.05 | 21.22*[z] | 18.84*** |
| | 22 | 99.29 | 83.76 | 74.00 | 63.82 |
| | 29 | 274.24 | 293.24 | 237.94 | 182.18*** |
| | 36 | 517.50 | 570.78 | 449.44 | 362.85 |
| | 43 | 896.95 | 791.56 | 653.93** | 521.65*** |
| Leaf fresh weight (g) | 15 | 0.96 | 1.05 | 0.83** | 0.69** |
| | 22 | 4.02 | 3.49 | 2.85* | 2.55* |
| | 29 | 10.92 | 12.17 | 10.09 | 7.71** |
| | 36 | 22.76 | 25.33 | 19.61 | 16.44 |
| | 43 | 39.77 | 35.62 | 29.93** | 25.56** |
| Leaf dry weight (g) | 15 | 0.120 | 0.131 | 0.108 | 0.091** |
| | 22 | 0.514 | 0.440 | 0.368* | 0.330* |
| | 29 | 1.595 | 1.742 | 1.399 | 1.028*** |
| | 36 | 3.414 | 3.600 | 2.872 | 2.203 |
| | 43 | 6.178 | 5.463 | 4.150** | 3.347** |
| Root fresh weight (g) | 15 | ---[y] | --- | --- | --- |
| | 22 | 0.434 | 0.465 | 0.305 | 0.332 |
| | 29 | 3.387 | 3.304 | 2.494** | 1.542*** |
| | 36 | 11.439 | 12.503 | 9.497 | 4.958 |
| | 43 | 25.154 | 28.503 | 18.430* | 13.545** |
| Root dry weight (g) | 15 | --- | --- | --- | --- |
| | 22 | 0.041 | 0.043 | 0.028 | 0.029 |
| | 29 | 0.306 | 0.309 | 0.231* | 0.140*** |
| | 36 | 1.029 | 1.250 | 0.959 | 0.502 |
| | 43 | 2.809 | 3.099 | 1.831*** | 1.250*** |
| Leaf/root dry weight | 15 | --- | --- | --- | --- |
| | 22 | 14.82 | 11.26 | 14.84 | 10.85 |
| | 29 | 5.99 | 6.40 | 7.02 | 8.89 |
| | 36 | 3.53 | 3.33 | 3.60 | 4.54 |
| | 43 | 2.30 | 1.98 | 2.47 | 2.91 |
| Leaf injury (%) | 15 | 0 | 0 | 2*** | 3*** |
| | 22 | 0 | 0 | 6*** | 11*** |
| | 29 | 0 | 1 | 5*** | 9*** |
| | 36 | 0 | 2 | 8*** | 13*** |
| | 43 | 0 | 2 | 5** | 6** |

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[z] *, **, *** - significantly different from the control at $P<0.10$, $P<0.05$, $P<0.01$, respectively.

[y] --- roots were not developed enough for accurate collection.

TABLE 2. Equations for fitted response surfaces (final models) and $r^2$ values.

| | $r^2$ |
|---|---|
| $\ln LA = -0.896 + 0.326(AGE) - 0.213(O_3) - 0.0034(AGE)^2 - 13.632(O_3)^2$<br>(FIG. 3) | 0.97 |
| $\ln LFW = 2.356 + 0.350(AGE) + 0.127g(O_3) - 0.0037(AGE)^2 - 14.539(O_3)^2$ | 0.97 |
| $\ln LDW = 0.208 + 0.357(AGE) - 0.102(O_3) - 0.0037(AGE)^2 - 14.084(O_3)^2$ | 0.97 |
| $\ln RFW = -9.253 + 0.482(AGE) + 8.242(O_3) - 0.0043(AGE)^2 - 36.856(O_3)^2 - 0.168(AGE \times O_3)$<br>(FIG. 2) | 0.96 |
| $\ln RDW = -4.565 + 0.474(AGE) + 5.084(O_3) - 0.0041(AGE)^2 - 27.850(O_3)^2 - 0.140(AGE \times O_3)$ | 0.97 |
| $\ln LFRTDW = 37.620 - 1.502(AGE) + 0.015(AGE)^2$<br>(FIG. 1) | 0.91 |

TABLE 3. Stomatal conductances (cm $s^{-1}$) of the abaxial surface of the first primary leaf of 'New Yorker' tomato seedlings; 1, 2, and 3 days following a 7 hour exposure to $O_3$.

| | Ozone concentration ($\mu l\ l^{-1}$) | | | |
|---|---|---|---|---|
| Day after exposure | 0.00 | 0.035 | 0.07 | 0.105 |
| 1 | 0.47 | 0.55 | 0.47 | 0.53 |
| 2 | 0.64 | 0.56 | 0.53 | 0.62 |
| 3 | 0.52 | 0.45**[z] | 0.45** | 0.45** |

[z] ** - significantly different from the control at $P<0.05$.

TABLE 4. Effects of a 7 hour $O_3$ exposure on soybean 'Amsoy-79' leaf and stem growth.

| | Ozone concentration ($\mu l\ l^{-1}$) | | | |
|---|---|---|---|---|
| Growth variable | 0.00 | 0.06 | 0.12 | 0.24 |
| Leaf area ($cm^2$) | 114 | 110 | 114 | 101**[z] |
| Leaf fresh weight (g) | 2.71 | 2.66 | 2.75 | 2.47* |
| Leaf dry weight (g) | 0.448 | 0.425 | 0.429 | 0.415 |
| Stem fresh weight (g) | 0.653 | 0.640 | 0.669 | 0.610 |
| Stem dry weight (g) | 0.076 | 0.076 | 0.079 | 0.074 |

[z] *, ** - significantly different from the control at $P<0.10$, $P<0.05$, respectively.

TABLE 5. Effects of a 7 hour $O_3$ exposure on the leaf growth of three wheat cultivars.

| Growth variable | Ozone concentration ($\mu l\ l^{-1}$) | | | |
|---|---|---|---|---|
| | 0.00 | 0.06 | 0.12 | 0.24 |
| Leaf area (cm²) | | | | |
| Abe | 17.46 | 18.92 | 18.17 | 19.69 |
| Arthur | 15.10 | 14.97 | 15.88 | 15.35 |
| Roland | 15.33 | 14.44 | 16.28 | 13.87 |
| Leaf fresh weight (g) | | | | |
| Abe | 0.487 | 0.544 | 0.482 | 0.538 |
| Arthur | 0.447 | 0.445 | 0.446 | 0.425 |
| Roland | 0.463 | 0.423 | 0.492 | 0.432 |
| Leaf dry weight (g) | | | | |
| Abe | 0.090 | 0.102* | 0.091 | 0.105** |
| Arthur | 0.086 | 0.084 | 0.083 | 0.082 |
| Roland | 0.093 | 0.079*** | 0.089 | 0.083** |

[z] *, **, *** - significantly different from the control at $P<0.10$, $P<0.05$, $P<0.01$, respectively.

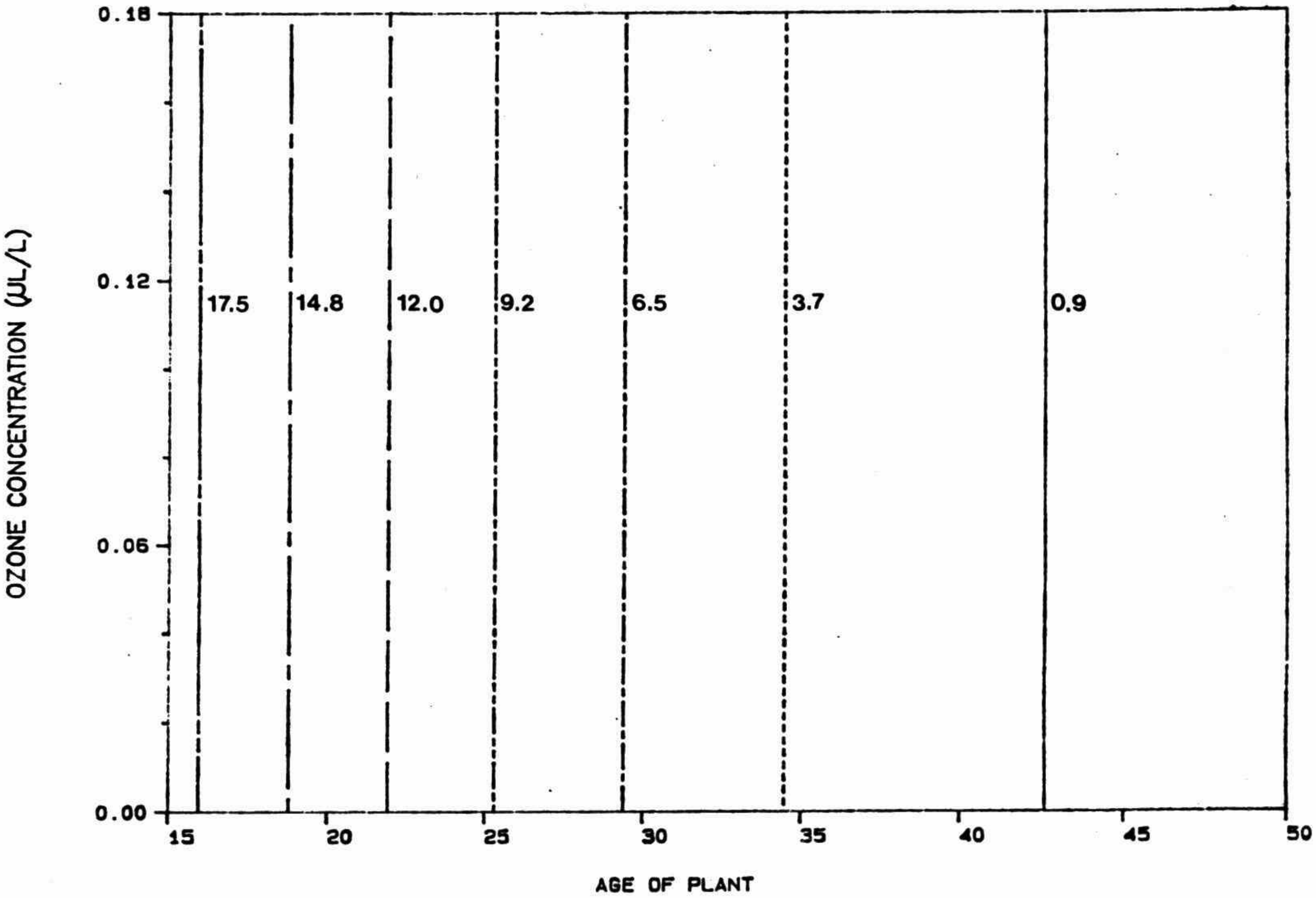

FIGURE 1. Contour plot illustrating the response of carrot leaf/root dry weight to weekly $O_3$ exposures throughout a growing season. The numbers on the contour lines indicate the fitted value for the line.

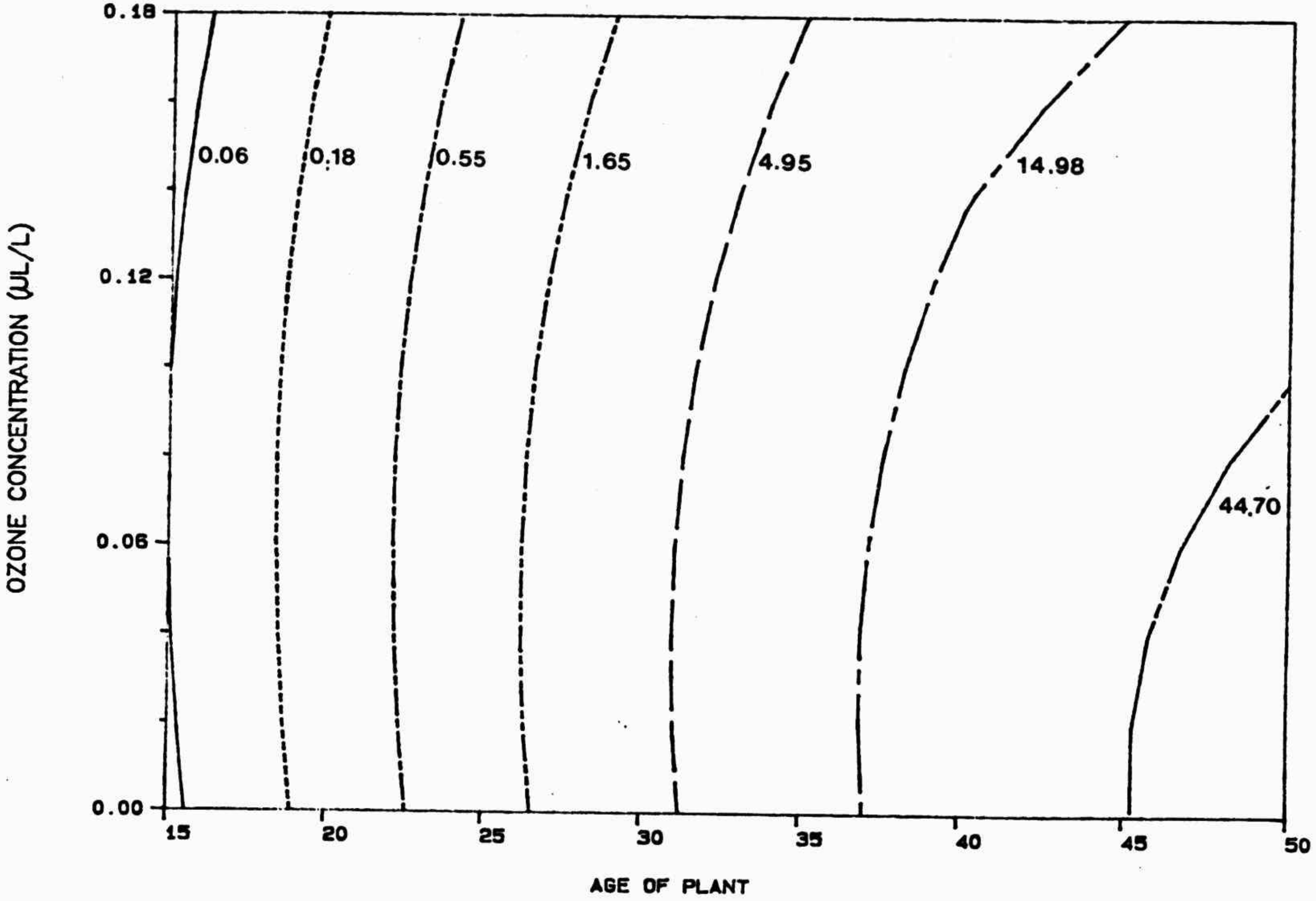

FIGURE 2. Contour plot illustrating the response of carrot root fresh weight to weekly $O_3$ exposures throughout a growing season. The numbers on the contour lines indicate the fitted value for the line.

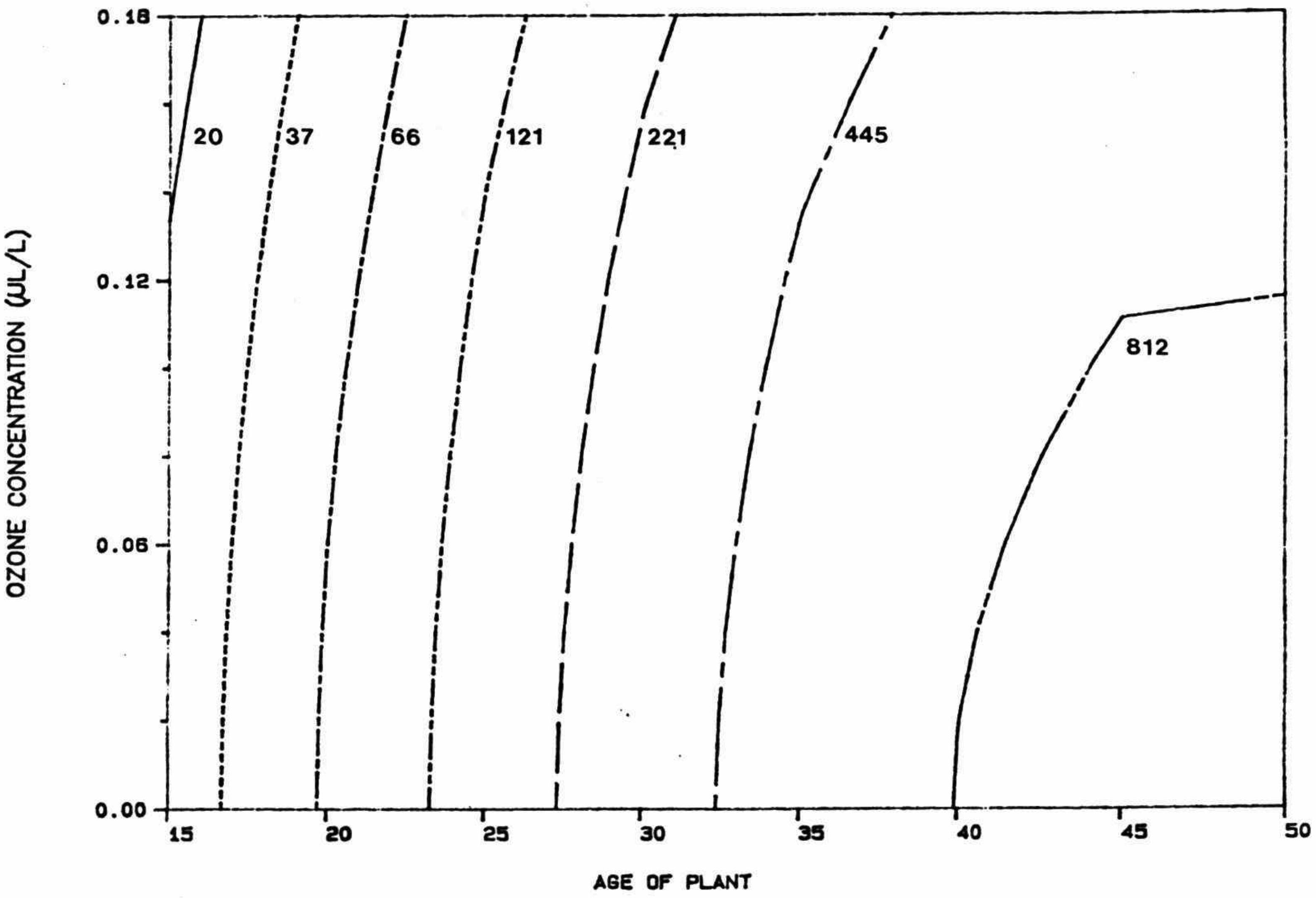

FIGURE 3. Contour plot illustrating the response of carrot leaf area to weekly $O_3$ exposures throughout a growing season. The numbers on the contour lines indicate the fitted value for the line.

# Selection of Ozone Insensitive White Bean Varieties Using Digitized Video Image Analysis

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## Abstract

Previous experiments using visual estimates of damaged leaf area following exposure of common bean (Phaseolus vulgaris L.) plants to ozone in growth chambers indicated that experienced evaluators are important in insuring rapid and consistent estimation of ozone damage. The objective of this study was to evaluate an alternative evaluation system that could be used by inexperienced technicians. A digitized video image analysis (DVIA) system was developed that evaluated the pixel brightness of video images of ozone--exposed bean leaves, and determined the mean and standard deviation of the resulting pixel brightness distributions for each leaf. Thirty-five near homozygous breeding lines were produced from crosses between the licensed white bean cultivars 'Seafarer', 'Kentwood' and 'Ex Rico 23', and ozone insensitive cultivars 'Gold Crop', 'French Horticultural Bean' and 'Midnight'. Fourteen-day-old seedlings of the 35 breeding lines and parental cultivars were exposed to 0.20 ppm ozone for 6 hours during each of 4 days in growth chambers. On day 5, injury of the two unifoliate leaves was evaluated using the visual Horsfall-Barratt scale and the DVIA system. The standard deviation of pixel brightness values was in good agreement with the damaged leaf area estimates (r=0.68). Cluster analysis of the standard deviation data placed 29 of 41 entries in the same sensitivity group as those based on visual estimates of damaged leaf area. Improvement in ozone insensitivity was most noticeable for breeding lines derived from the early white bean cultivars 'Seafarer' and 'Kentwood'.

## Introduction

White bean, also called navy or pea bean, is the predominant common bean (Phaseolus vulgaris L.) type grown in Ontario. About 40,000 hectares of white beans are cultivated annually in southwestern counties of the Province, and generate a farmgate income of approximately $40 million. The currently licensed and recommended white bean varieties are susceptible to leaf damage following episodes of high atmospheric ozone concentration during the growing season. Ozone-induced leaf damage, or bronzing, has been estimated to reduce white bean yields by more than 30% (Hofstra et al., 1978). A 1984 report by the Ontario Ministry of the Environment cited white beans as suffering a higher percentage loss from ozone than other crops studied: a 12% loss in region 5 and 7% loss in region 4 (Linzon et al., 1984). The monetary value of these losses was estimated at up to $1,185,000. Because of this culture's reliance on fossil fuels, it seems unlikely that ozone levels will decline in the short term.

During the development of new white bean cultivars for commercial use, thousands of genetically different plants are generated, and those with the best combinations of the desired agronomic and quality attributes are selected. In our breeding program, ozone damage has routinely been evaluated by estimating the percentage of seedling leaf area damaged by exposure to ozone in growth chambers using the Horsfall-Barratt scale. In this 12 division scale, divisions representing damaged leaf areas near 50%, where the eye's acuity is lower, cover a wider range of disease severity than divisions representing damaged leaf areas near 0 or 100%. (Horsfall and Barratt, 1945). Our experiences with the Horsfall-Barratt scale indicate that well trained evaluators are important in insuring rapid and consistent estimation of ozone damage. In addition, aprolonged evaluation session can cause fatigue in the evaluator which results in decreased speed and accuracy.

Video image analysis offers an alternative electronic method of assessing plant disease severity. In this technique, a video camera transmits the image of a leaf to a video digitizing circuit board installed in a microcomputer. The video board divides the image into 60000 pixels, assesses the brightness of each pixel, and assigns each a brightness value ranging from 0 (black) to 255 (white). In previous video image analysis systems, the ratio of pixels with brightness values representing diseased leaf tissue to the total number of pixels corresponding to healthy and diseased leaf tissue was used to compute the percentage of damaged leaf area (Lindow, 1983). Successful operation of the video image analysis system required distinct differences in brightness among healthy plant tissue, diseased tissue, and background. In preliminary attempts at digitized video image analysis of ozone damaged leaves, I had little success at obtaining distinct brightness differences between damaged and healthy leaf tissue. I observed, however, that the frequency distribution of pixel brightness from healthy leaves differed from those of ozone-damaged leaves. Healthy leaves tended to produce frequency distributions with smaller standard deviations than the distributions from ozone damaged leaves. The mean brightness of ozone-damaged leaves also appeared to be somewhat higher or lower than the mean brightness of healthy leaves.

Our goal was to develop an alternative means of evaluating ozone damage induced in a large number of white bean breeding lines that did not require experienced operators or induce fatigue, yet was rapid and accurately assessed genotypic differences in ozone insensitivity. The objective of this study was to determine whether the mean and standard deviation of the frequency distribution of pixel brightness from video images of ozone-exposed common bean leaves could be used to identify ozone insensitive breeding lines.

## Materials and Methods

Thirty-five white bean breeding lines were developed from crosses between the ozone insensitive common bean cultivars 'French Horticultural Bean', 'Midnight' and 'Gold Crop' and ozone sensitive white bean cultivars 'Seafarer', 'Ex Rico 23' and 'Kentwood', followed by selection for plant and seed type in the $F_5$ and $F_6$ generations. Two Fourteen-day-old seedlings at the unifoliate stage of these thirty-five lines and the six parental cultivars were screened for ozone sensitivity in each of four growth chambers in Spring, 1986. One of the chambers received no ozone while the others received 0.20 ppm ozone for 6 hours during each of 4 consecutive days. On day 5, injury of the two unifoliate leaves of each plant was rated using the Horsfall-Barratt scale and the Digitized Video Image Analysis system. Only the Digitized Video Image Analysis system was used to rate the plants from the chamber receiving no ozone. Prior to digitization, the leaves were detached from the plant and placed on a black velvet background with illumination from a small fluorescent desk lamp. The DVIA system used a Panasonic WV 1330 video camera interfaced with a Techmar Video Van Gough expansion board installed in an IBM Portable PC. User-written software computed the frequency of pixels with each brightness value, and determined the mean and standard deviation of the pixel brightness distribution for each leaf image. The difference in mean and standard deviation between ozone exposed and unexposed leaves of the same genotype was also computed. Horsfall-Barratt scores and video image data were evaluated using an Analysis of Variance. Log transformations of the Horsfall-Barratt and standard deviation data were required to meet the assumptions of the Analysis of Variance. Where significant differences existed, entry means were separated based on a K-means cluster analysis where means were separated into four groups.

## Results

Analysis of variance of the Horsfall-Barratt scores of damaged leaf area and the pixel brightness distribution statistics indicated that breeding line differences were significant. Damaged leaf area was most closely correlated with the standard deviation of the pixel brightness distribution (Table 1). Damaged leaf area was not, however, correlated with the mean of the brightness distribution. Differences between exposed and unexposed leaves as measured by the brightness distribution statistics were not more closely associated with estimated damaged leaf area than standard deviation of the exposed leaves alone.

Of the parental white bean cultivars, 'Seafarer' was grouped with the most ozone-sensitive breeding lines, 'Kentwood' was placed in an intermediate group, and 'Ex Rico 23' was placed in the least sensitive group (Table 2). Two of the common bean parents used as donors of ozone insensitivity, 'Midnight' and 'French Horticultural Bean', were also grouped with the least sensitive group. However, the supposedly ozone-insensitive parent 'Gold Crop' was placed with breeding lines of intermediate sensitivity.

Twenty-two breeding lines were less ozone sensitive than their white bean parent. Six were more sensitive, and seven were placed in the same group as their white bean parent. Improvement was most noticeable for breeding lines derived from the early white bean cultivars 'Seafarer' and 'Kentwood'. Five lines derived from 'Ex Rico 23' maintained the low sensitivity of the parent line. Cluster analysis of the standard deviation data placed 29 of 41 entries in the same sensitivity group as those based on visual estimates of damaged leaf area.

## Discussion

The standard deviation of the pixel brightness distribution of ozone-exposed leaves was an acceptable indicator of ozone sensitivity of the various breeding lines. Standard deviation was highly correlated with visual estimates of damaged leaf area. The lack of perfect correlation between damaged leaf area and standard deviation did not necessarily reflect flaws in the DIVA system, since the damaged leaf area was estimated visually, and was itself subject to error. Low standard deviations indicated ozone insensitivity, while high standard deviations indicated greater sensitivity. Leaves from ozone insensitive plants had a uniformly green color, which produced a distribution with a high number of pixels in a small range of brightness values. Damaged leaves displayed several different symptoms including chlorosis, necrosis and bronze stippling in addition to healthy green tissue, which resulted in distributions with pixel brightness values spread over a wider range. Increased tissue damage did not substantially alter the mean of the brightness distribution, probably because some symptoms such as chlorosis produced brighter pixels upon digitization than healthy green tissue, while other symptoms such as necrosis caused darker pixels.

A comparison of leaf brightness distributions of exposed and unexposed leaves did not improve the ability of the system to distinguish differences among the breeding lines since the leaves of genotypes used in this study showed little variation in their brightness distributions prior to ozone exposure.

The visual and DVIA systems could potentially differ in how the chlorotic, necrotic and bronzing symptoms are interpreted. Three leaves with equal areas of damage, but each differing in the primary symptom should be ranked equally by an experienced evaluator using the visual method, but may be ranked differently by the DIVA system since the brightness of the symptoms differ. Because plants selected based on this ozone sensitivity screen would be those with no damage symptoms, this difference in evaluation would not be important in a breeding program. However, in experiments quantifying the genetic variation for ozone insensitivity, the difference in interpretation could cause differences in heritability estimates. In cases of extremely severe ozone damage where the symptom is uniform across the leaf, the resulting standard deviation would be low, and the leaf rated as insensitive. In this case, a rating formed from a combination of standard deviation and mean might be useful.

The primary advantage of this digitized video analysis system is its ease of use by inexperienced personnel, and the consistency of the evaluations regardless of the duration of the evaluation session. In a preliminary study, an inexperienced technician successfully collected brightness data on leaves after a 30 minute training session. Data collection using the video system took approximately as much time as the visual analysis. Each leaf was digitized and saved to a file in approximately 15 seconds. Positioning the leaf on the background and entering an identification code into the computer required more time than the digitizing and file manipulation steps.

The system may have applications to field studies where a portable video tape recorder is used to collect the images of the tissues to be evaluated.

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Lindow, S. E. 1983. Estimating disease severity of single plants. Phytopathology 73:1576-1581.

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Table 1. Correlations among visual ratings and digitized video image pixel brightness distribution statistics.

| | Mean | Δ mean | Standard deviation | Δ Standard deviation |
|---|---|---|---|---|
| Damaged leaf area | 0.19 | 0.21 | 0.68 | 0.65 |
| Mean | | 0.65 | 0.16 | 0.10 |
| Δ mean | | | 0.15 | 0.24 |
| Standard deviation | | | | 0.93 |

Table 2. Mean damaged leaf area based on visual observations, and mean and standard deviation of digitized video image pixel brightness distributions.

| Breeding line | Pedigree | Damaged leaf area | Pixel brightness | |
|---|---|---|---|---|
| | | | Mean | Standard deviation |
| | | % | | |
| 19 | F[†] x S | 1.2 a[‡] | 138.5 c | 26.5 a |
| 9 | E x G | 2.0 a | 131.4 a | 31.1 b |
| 11 | F x S | 2.3 a | 139.9 c | 27.0 a |
| 30 | (E x G) x M | 2.3 a | 132.4 a | 25.4 a |
| 1 | S x G | 2.9 a | 133.8 b | 27.2 a |
| 3 | S x G | 2.9 a | 136.0 b | 26.4 a |
| 6 | K x G | 2.9 a | 136.1 b | 26.8 a |
| 12 | F x S | 2.9 a | 137.2 b | 26.0 a |
| 14 | F x S | 3.1 a | 139.2 c | 27.8 a |
| 27 | (K x F) x M | 3.7 a | 133.2 a | 29 0 a |
| | **Midnight** | 4.5 a | 128.1 a | 26.7 a |
| 28 | (E x G) x M | 4.7 a | 142.1 c | 28.6 a |
| | **Ex Rico 23** | 5.1 a | 136.8 b | 26.4 a |
| 20 | F x S | 5.3 a | 132.1 a | 28.1 a |
| 21 | S x G | 5.9 a | 148.5 d | 31.7 b |
| 8 | E x G | 6.3 a | 136.1 b | 27.3 a |
| 4 | S x G | 6.4 a | 136.9 b | 27.4 a |
| 31 | (E x G) x M | 6.8 a | 135.4 b | 25.9 a |
| 5 | K x G | 7.4 a | 143.2 c | 28.0 a |
| 24 | K x F | 8.0 a | 133.7 b | 27.8 a |
| 15 | F x S | 8.4 a | 137.4 b | 28.8 a |
| 22 | S x G | 8.5 a | 142.5 c | 27.1 a |
| 23 | S x G | 8.6 a | 135.6 b | 30.7 b |
| | **French Horticultural** | 8.8 a | 129.5 a | 27.1 a |
| 13 | F x S | 9.5 a | 137.8 b | 27.7 a |
| 17 | F x S | 10.1 a | 137.6 b | 27.4 a |
| 7 | K x G | 12.0 a | 130.8 a | 30.5 b |
| 10 | E x G | 13.8 b | 137.6 b | 28.1 a |
| | **Kentwood** | 15.6 b | 138.3 c | 31.7 b |
| 2 | S x G | 17.2 b | 138.9 c | 27.1 a |
| 29 | (E x G) x M | 18.0 b | 148.4 d | 28.8 a |
| 25 | K x F | 18.9 b | 147.5 d | 31.8 b |
| 18 | F x S | 19.7 b | 142.0 c | 30.0 b |
| 26 | (K x F) x M | 22.1 b | 133.8 b | 33.2 c |
| 16 | F x S | 24.9 b | 140.1 c | 28.6 a |
| | **Gold Crop** | 27.7 c | 134.3 b | 33.9 c |
| 34 | G x E | 33.7 c | 140.0 c | 32.5 b |
| 33 | (G x E) x M | 34.0 c | 138.0 b | 35.3 c |
| 35 | K x F | 35.1 c | 141.9 c | 30.6 b |
| | **Seafarer** | 53.3 d | 135.7 b | 34.0 c |
| 32 | (K x F) x M | 60.0 d | 135.2 b | 40.9 d |

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[†] F = French Horticultural Bean, S = Seafarer, E = Ex Rico 23, G = Gold Crop, M = Midnight, K = Kentwood

[‡] groups established by k-means cluster analysis

# Nickel Carbonyl Ambient Monitor

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M3J 1P3

## Nickel Carbonyl Ambient Monitor

### Introduction

The purpose of the contract was to produce an instrument capable of measuring ambient levels of Nickel Carbonyl down to the Ontario Provincial standard of 0.5 $\mu gm^{-3}$ (70 ppt).

The analytical technique that was chosen uses the chemiluminescence which results when Nickel Carbonyl is reacted with ozone.

## General Theory of Operation

The gas-phase reaction of Nickel Carbonyl ($Ni(CO)_4$) and ozone ($O_3$) in the present of carbon monoxide (CO) produces a characteristic luminescence with an intensity linearly proportional to the concentration of Nickel Carbonyl. Specifically,

$$\mathrm{Ni(CO)_4 + O_3 \qquad NiO + products}$$

$$\mathrm{NiO + CO \qquad Ni + CO_2}$$

$$\mathrm{Ni + O_3 \qquad NiO^{*} + O_2}$$

Light emission results when electronically excited NiO molecules decay to lower energy states.

The ambient air sample enters the instrument through a single flow control capillary. The sample is then mixed with carbon monoxide and ozone and admitted to the reaction chamber where the chemiluminescence is measured. An output signal is continuously generated which records the level measured.

## Instrument Modification

The Thermoelectron Corporation (TECO) markets a Nickel Carbonyl monitor, model 14N, but the specifications indicate it is not sufficiently sensitive for Ministry requirements. The detection limit is listed as $0.7 \mu g m^{-3}$(.1ppb) Thus for this contract this instrument would have needed to be modified. The Nickel Carbonyl instrument is a modified model 14B/E $NO/NO_x$ instrument. Since modifications would have been required to the Nickel Carbonyl instrument, it was decided it would be more cost effective to purchase the $NO/NO_x$ instrument and modify it to measure Nickel Carbonyl at the required sensitivity.

The following modifications were made to the commercial $NO/NO_x$ instrument:

a) The volume of the reaction vessel was increased from 10 to 100 cubic centimeters. This increases the number of Nickel atoms being viewed by the photomulitplier and thus increases the sensitivity.

b) The air flow through the system was increased which increases the number of Nickel atoms passing through the cell and thus the sensitivity.

c) The red filter in front of the photomultiplier was replaced with an Andover 023FG11-50 blue filter. This was selected to maximize transmission of the $NiO^*$ chemiluminescence while rejecting the $NO_2^*$ chemiluminescence from NO and the $FeO^*$ chemiluminescence from $Fe(CO)_5$. In this way the selectivity toward $Ni(CO)_4$ was assured.

d) An additional plumbing line to allow addition of CO was included. It was found necessary to include a needle value on this line to optimize instrument sensitivity. A scrubber containing iodine and activated charcoal was included on this line to remove any carbonyl in the carbon monoxide cylinder. (In the course of this work it was found that normal CP grade carbon monoxide contains extremely variable amounts of carbonyls. We recommend a high purity grade of CO in an aluminum cylinder be used for this instrument.)

e) The sample and hold electronics of 14 B/E is now redundant and has been bypassed. The signal from the photomultiplier now passes through an amplifier, a temperature compensation board, and switch is then filtered and appears as the recorder output. None of the original control electronics is used.

f) While running instrument tests the photomultiplier tube envelope fractured. Thus we had to replace this tube. A direct replacement was quoted as being $3,000. The high cost is related to the low quantum efficiency of photomultipliers in the red end of the spectrum. However, for this instrument we block the red end of the spectrum. As a result a cheaper photomultiplier could be used. We purchased a Hamamatsu Model R329 tube for $300 U.S. which could be used without modifying the instrument. In addition this tube has a far lower dark current at room temperature and while it was higher than the cooled tube the improvement was so small we decided to disconnect the thermoelectric cooler.

g) The final modification to the instrument was to replace the supplied pump with a rotary vacuum pump. It by found that using the diaphram pump that the relationship between signal and Nickel Carbonyl concentration was not linear. It was found that by lowering the cell pressure linearity was achieved. This necessitated the replacement of the diaphram pump with a rotary vacuum pump. It should be noted here that ozone reacts with rotary pump oil in making it very thick and potentially capable of seizing the pump.

Two precautions need to be taken to lengthen the life of the pump. Firstly the TECO ozone destruction catalyst should be placed between the instrument and the pump, not after the pump as with the 14B/E. Secondly the pump oil should be replaced regularly.

## Instrument Tests

Before delivering the instrument many tests were performed to ensure it met the contract standards. The two instrument parameters that were of most concern were sensitivity (and its associated parameter, detection limit) and linearity. We also examined some parameters related to the reliability of the instrument. The bulk of the tests however related to the development of a calibration and sampling protocol.

## Instrument Linearity

This section of the work was expected to be relatively straighforward. The mechanism for this reaction is well known and predicts a linear relationship between signal and Nickel Carbonyl concentration. Varying parameters such as air flow, ozone flow and reaction cell pressure would be expected to change the sensitivity (the signal per unit of concentration) but a linear relationship would be expected for any parameter combination. (This is not strictly true for low ozone concentrations but this case is never encountered so was not considered)

Figure I shows the linearity check run for an early geometry and using the TECO supplied pump. Non linearity is clear. For a halving of the Nickel Carbonyl concentration the signal drops by a factor of just over 2 whereas halving the concentration again drops the signal by an order of magnitude.

We found both the signal magnitude to increase and the signal concentration relation to more closely approach linearity as the air ozone and the carbon monoxide were premixed closer to the reaction vessel. The prereactor in particular showed some irreproducible behaviour and since it appeared not to be necessary for NO signal rejection it was removed. We tried many combinations of flows and plumbing schemes but it was not until we lowered the reaction vessel pressure that linear behaviour became reproducible. Figure II shows the same plot as Figure I but for the final system geometry and shows the instrument has a linear relationship between signal and Nickel Carbonyl concentration.

Note:

It is important to understand how the linearity tests were performed. A constant ambient air flow was doped with varying flows from the Nickel Carbonyl cylinder. We used Hastings mass flowmeter for the flow measurements but found that the heat of the flowmeter was sufficient to decompose the $Ni(CO)_4$. Thus the protocol was to set the Nickel Carbonyl flow and measure it using the flowmeter. The signal on the instrument was zero at this time. We then turned the flowmeter off and as it cooled the signal rose. After 15 minutes the signal stabilized and this value was used. The flowmeter was turned on again to ensure the flow had not changed. This procedure was followed for all flows, i.e. for all concentrations.

## Sensitivity and Detection Limit

Once linearity was assured it was then possible to determine the sensitivity and detection limit. Using the scale remaining from the $NO/NO_x$ application we found the sensitivity to be 4 concentration units for 1 ppb of Nickel Carbonyl. This gives a sensitivity of 4 concentration units per ppb. The peak to peak noise on the background is 0.02 concentration units corresponding to <10 ppt of Nickel Carbonyl. Thus using the instrument with a chart recorder output the detection limit is 10 ppt and could be improved using signal averaging.

## Calibration Sources

a) Permeation Source.

The initial attempt at an in situ calibration source was a permeation device. In this case Nickel Carbonyl in a container at 35°C was separated from the air stream going to the instrument by a wafer of teflon. The concentration gradient across the wafer then drives diffusion, or permeation, of Nickel Carbonyl through the wafer. A constant mass of Nickel Carbonyl would pass through the wafer which could be fed to the instrument for calibration. We used wafer as low as .010" thick but were unable to get sufficiently high permeation rates for a sensible calibration source.

## Diffusion Source

The diffusion source is similar in principle to the permeation source except that the wafer is replaced by a short length of capillary tubing. This worked much better than the permeation source but was found to be more variable than could be tolerated. On opening the source we found nickel deposits on many occasions showing Nickel Carbonyl decomposition in the capillary which changes the geometry and thus the diffusion rate. The diffusion device also showed variable rates when the unit was moved showing the effects, we believe, of Nickel Carbonyl being splashed within the device.

## Calibration cylinders

At the beginning of this project we attempted to find a supplier of gas cylinders of Nickel Carbonyl at part per million mixtures. We were successful but believed they were overly expensive and since we expected the permeation device to work we did not pursue the option.

The failure of permeation and diffusion devices left us with only the cylinder as an option. The long lead time and cost of the commerical product led us to make our own cylinders. We had a passivated aluminum cylinder pressure tested and a new valve fitted. In this we made mixtures of Nickel Carbonyl in Nitrogen by pumping the cylinder to $10^{-5}$ Torr, adding 10's of Torr of $Ni(CO)_4$ and filling the cylinder to over 1000 psi with nitrogen. We then calibrated these cylinders regularly. We have seen substantial decay of the standard. We feel this is largely due to decomposition within the cylinder. This could be improved by making the mixture in carbon monoxide rather than nitrogen, a procedure which is done by the commerical supplier but which we were reluctant to do.

We thus recommend the purchase of standard Nickel Carbonyl standards in carbon monoxide but in light of our experience we recommend regular calibrations until confidence in the stability of the standard is established.

## Calibration of Nickel Carbonyl Tanks

To determine the concentration of Nickel Carbonyl in cylinders an independent absolute analysis system was developed. The principle is to take on some of the gas mixture and collect the Nickel Carbonyl in a solution. The nickel is oxidized to $Ni^{II}$ and complexed with diethyl dithiocarbamate to produce a product with a very strong UV absorption spectrum with a known extinction coefficient. The concentration of the complex is determined by UV absorption allowing the determination of the amount of Nickel Carbonyl in the original sample. By measuring the flow of the gas mixture and the time taken to collect the sample the concentration of Nickel carbonyl can be determined.

## Procedure

The Nickel Carbonyl tank is connected to a bubbler containing approximately 30 ml of 1:1 nitric acid. The output from the bubbler is connected to a flowmeter to determine the flow (we used a soap bubble meter). The gas mixture was flowed through the bubbler at a rate of about 50 sccm for 30-60 minutes. The nitric acid dissolved the Nickel Carbonyl and oxidized it to $Ni^{II}$. The sample was washed from the bubbler into a 50 ml volumetric flask for analysis as $Ni^{II}$.

An aqueous solution of 0.1% sodium diethyldithiocarbamate (BDH AnalaR grade) was prepaed with water which was purified by double-distillation over $KMnO_4$ followed by passage through a Barnstead organic removal cartridge and two Barnstead mixed resin ultrapure cartridges. This solution was extracted with successive portions of carbon tetrachloride (BDH Assured, ACS grade) until the $CCl_4$ layer was colourless.

The pH of the 1:1 nitric acid solution was adjusted to the proper range for complexation with diethyldithiocarbamate by the addition of ammonium hydroxide (ACS grade). The pH was tested with colorpHast Indicator sticks to ensure that it was between 8.5 and 9.0. Once the proper pH was achieved, 2 mL of the sodium diethyldithiocarbamate was added and the solution was transferred to a separatory funnel. Two milliliters of carbon tetrachloride was added to the solution followed by vigorous shaking for at least two minutes. The $CCl_4$ layer was then collected in a 10 mL volumetric flask. The extractions were continued until the volumetric flask was filled to the mark. The electronic spectrum of this solution was then recorded over the range 300 to 500 nm using quartz cells and a Hitachi-Perkin Elmer Microprocessor model 340 spectrometer.

## Discussion

The number of moles of nickel present in each sample was determined by the expression

$$\text{moles of Ni} = 2.84 \times 10^{-7} A_{328nm} \quad (1)$$

based on the extinction coefficient given in Chilton's article [1]. The concentration of $Ni(CO)_4$ could then be determined by dividing this value by the product of the flow rate and time of flow.

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[1] J.M. Chilton, Anal. Chem., 25, 1274-1275 (1953).

## Conclusion

An instrument capable of measuring Nickel Carbonyl concentrations in the air down to the Ontario standards has been developed.

It has been shown to have a detection limit of 10 ppt.

A protocol for in-situ calibration from a cylinder containing Nickel Carbonyl has been developed, as has a technique for calibration of cylinders.

## Acknowledgement

We are indebted to the Ontario Ministry of the Environment for support of this work and would like to acknowledge useful discussions with W.C. Tam, A. Szakolcai of the Ministry's Air Resources Branch and R. Potvin of Northeast Region.

V. Antunes and P. Sum ably performed much of the experimental work.

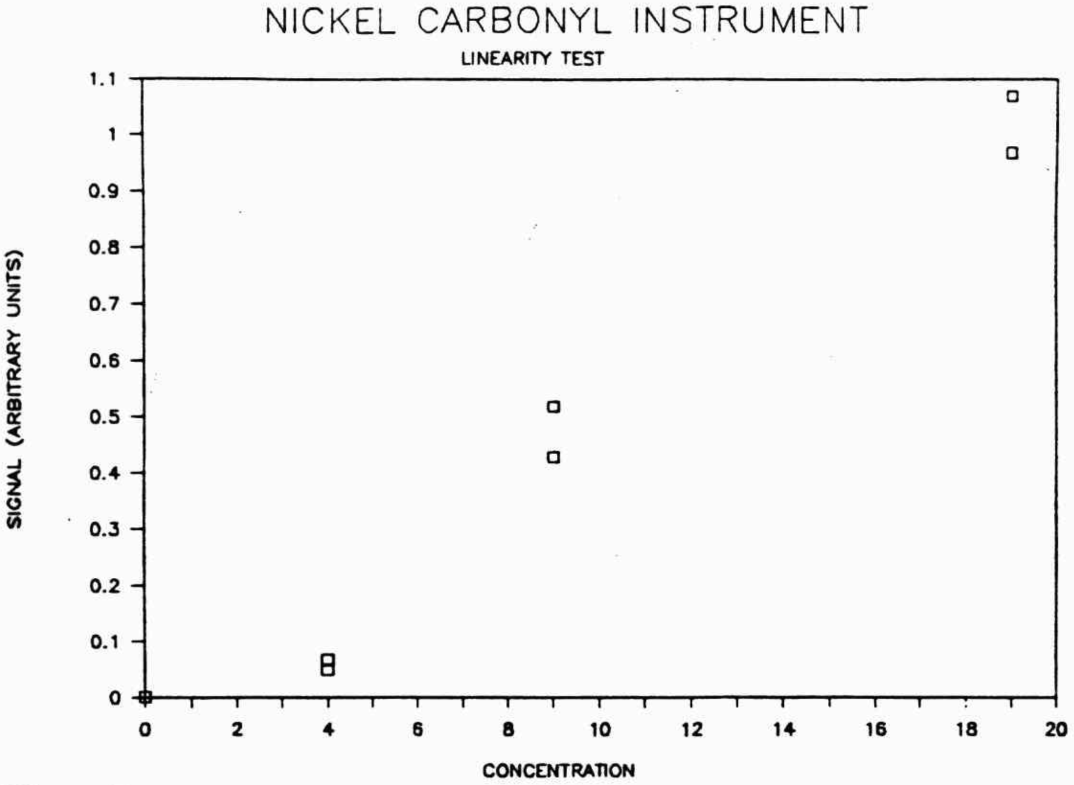

**Figure I**

**Nickel Carbonyl instrument response showing strong non linearity**

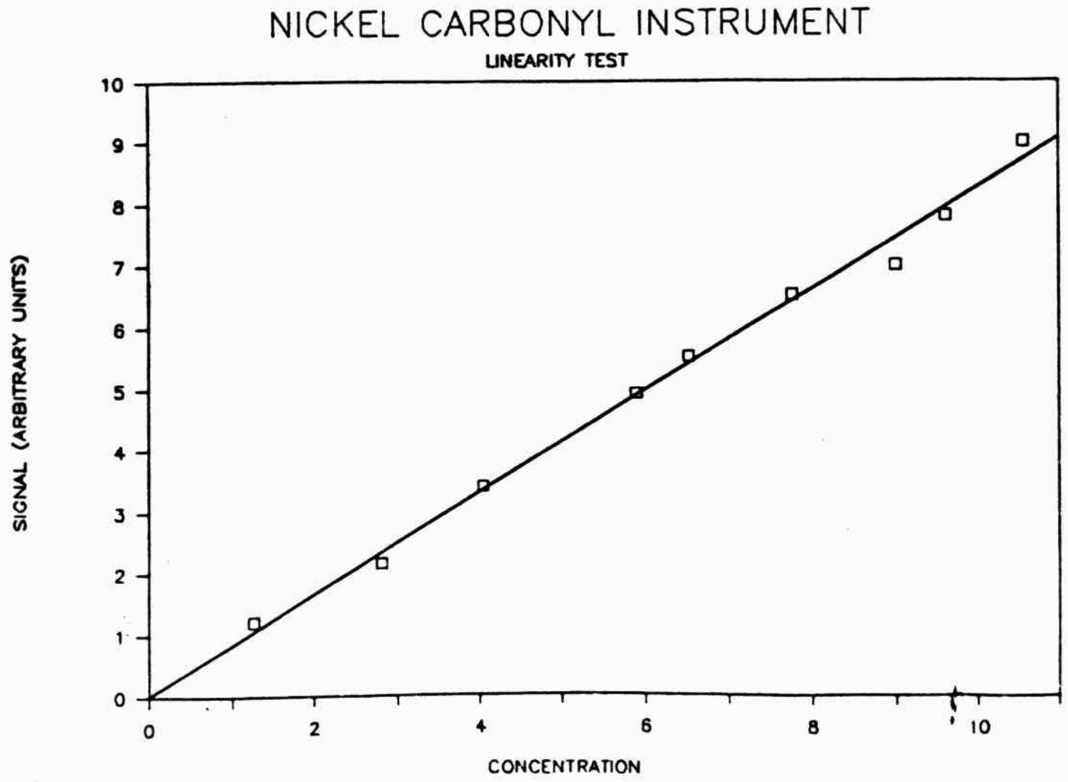

Figure II

Nickel Carbonyl instrument response using final configuration - showing linear response

# "ELEMENTAL COMPOSITION OF AIR PARTICULATES INSIDE AND OUTSIDE A BUILDING".

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## Background

At last year's OME conference, a paper was presented which laid down much of the ground work necessary for this report.[1] In this paper the results from the first week of a two week filtering period in June 1985 were presented. In the present paper, the results from the second week of this filtering period are analyzed. As well, some preliminary results from a recent 3 week filtering period in Feb-March 1986 are discussed.

## Introduction

This project is concerned with indoor air quality and the relationship between indoor and outdoor particulate air pollution levels. Five filtering stations have been set up in and around a high school in Kingston Ontario in order to measure these effects. Three of the filters were placed in separately ventilated areas within the school, and the other two which were equipped with meteorological recording devices were placed outside, one beside the school near a busy intersection and the other in a nearby residential backyard.

The filter used was a moving 0.4μm nucleopore filter which yields a time resolution of 2 hours. These filters were analyzed using proton induced X-ray emission (PIXE) on the Queen's Van de Graaff accelerator. Elements commonly detected are Al, Si, S, Cl, Ca, Ti, Mn, Fe, Zn, Pb and Br with Na, K, Cr, Cu and Sr being detected less frequently. Our detection limits for the elemental concentrations in air are typically less than 0.03μg/m$^3$. The reader is referred to last years OME conference proceedings (ref 1) for a more detailed description of apparatus and analytical techniques.

## General Results

Using factor analysis to organize the detected elements into groups, it was observed that the elements could generally be collected into four main classifications:

1) Dust -- This factor is responsible for about 80% of the total suspended particulate matter analyzed for the indoor filters, and consists of Si, Fe, Al, Ca, Cl, Mn, Cu, Zn and Sr. Of these, Si, Fe, Al, Ca, Mn and Sr are found in local soil samples, while the other elements (Cl, Cu and Zn) are observed mainly in indoor dust.

2) Industrial pollutants -- Sulphur is the only observed element that can be attributed to pollution by industrial sources.

3) Automotive pollutants -- Pb and Br are elements associated with automotive emissions, and are found at all sampling sites to be in approximately the ratio 5:1 which is typical of car exhaust.

4) Trace elements -- These are the elements which usually appear in concentrations close to our

detection limits. For these elements, the noise introduced by statistical fluctuations in the sampling and analysis makes categorization difficult. These elements include Na, K, Cr, Ti, Mn, Cu, Zn and Sr.

Shown in Figure 1 are example plots of the element scans for the S3 (main office) filter location in June 1985. Note the lower concentrations of the dust elements on the weekends of June 8-9 and June 15-16.

At times, in addition to these four categories, other factors can be introduced by specific human activities indoors. For example, one episode involving high indoor concentrations of Ti was attributed to spray-painting, and another with high Cu and Fe levels is thought to result from a hair spray used when one of the filters was placed in the hairdressing room of the school.

In this report, we present a model for the indoor concentrations of elements belonging to the first three categories. The formula that will be used has the following general form:

$$C_i = c_1 C_o + c_2 f(t) \qquad 1)$$

where $C_i$ = Indoor concentration of an element.
$C_o$ = Outdoor concentration of element.
$f(t)$ = A time varying indoor source function.
$c_1, c_2$ = Empirically determined constants.

Major progress has been made since last year's report by introducing a time varying function used to model the amount of each element injected into the air by human activity within the building. The following three sections of this report will be dedicated to developing the form of f(t) and determining the values of $c_1$ and $c_2$ for each of the three groups of elements discussed previously.

### Group 1 -- Dust

For this factor, the term $c_2 f(t)$ has been introduced due to the large amounts of dust which are stirred up by human activity within a building. An estimate of the function f(t) has been made by assuming that the activity within the building is constant between the hours of 8:30 and 16:00 on weekdays, with no activity otherwise. Clearly, counting the number of people in each room at a given time would give more accurate results, but this data was not available.

Assuming that the rate at which dust is injected into the air is proportional to the activity within the building, and that the airborne dust is removed from the room or settles out of the air with a mean residence time of 2 hours, then the concentration of dust in the air on a weekday takes on the following form (t in hours):

$$\begin{aligned} f(t) &= 0\ , && 0{:}00 < t \le 8{:}30 \\ &= 1.0241*\{1-\exp[-(t-8{:}30)/2]\}\ , && 8{:}30 < t \le 16{:}00 \\ &= \exp[-(t-16{:}00)/2]\ , && 16{:}00 < t < 24{:}00 \end{aligned}$$

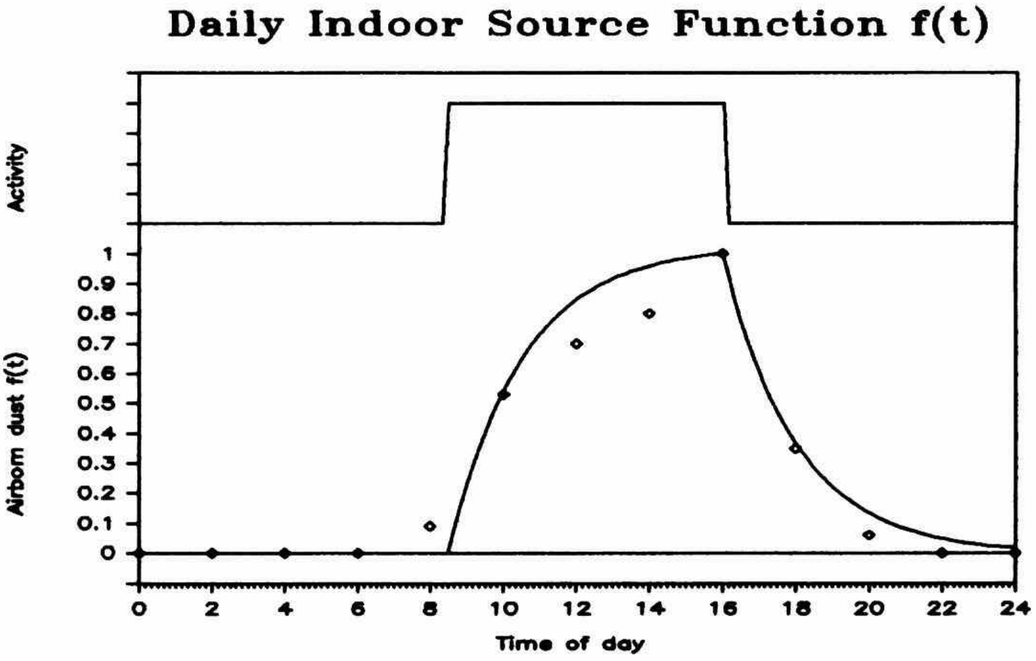

Figure 2 -- Graph of human activity and f(t) used in modelling indoor source term.

On the graph, the theoretical curve for f(t) is given by the solid line, and the experimental points correspond to an average of the indoor dust factor scores for all indoor samplers for each weekday between June 3 and June 17/85. The residence time of 2 hours was chosen so that the theoretical curve best fit the experimental data. It can be seen that f(t) is representative of the average relative amount of dust suspended indoors on weekdays. During weekends, there is assumed to be no activity.

Using multiple regression, the data from each of the indoor sampling sites was modelled by combining the activity function and the elemental concentration as measured in the schoolyard using formula 1). For the soil elements, the coefficient $c_1$ is expected to be the lowest since natural particles typically have larger diameters than particles from anthropogenic sources, and the filtering efficiency of the ventilation system is higher for the larger particles. Table 1 gives the derived values for $c_1$ and $c_2$ for Al (considered here to be representative of the natural dust elements). The errors given represent only those in the multiple regression fit, and not errors in the data itself. Also given is a correlation coefficient between the resulting scan and the experimental elemental scan. (See figure 3 for a plot of the fit obtained for S3.)

Table 1

| | $c_1$ | $c_2$ ($\mu g/m^3$) | corr |
|---|---|---|---|
| S1 - Business room | 0.15 ± 0.02 | 0.09 ± 0.02 | 0.468 |
| S2 - Modelling room | 0.12 ± 0.02 | 0.09 ± 0.02 | 0.301 |
| S3 - Main office | 0.15 ± 0.02 | 0.25 ± 0.02 | 0.727 |

The activity coefficient $c_2$ is largest for the office. One reason for this is that the office is carpeted, and the carpet serves as a dust reservoir from which the soil elements are repeatedly suspended. For the other rooms, the activity was not as uniform from day to day, so the fits to these data were not as good as for the office (this is illustrated by the correlation coefficients shown). The indoor/outdoor ratio $c_1$ was similar for all rooms. Any variations can be attributed to the different ventilation systems of the three rooms. In June, this coefficient should be higher than during the other filtering period of late February because of open windows and doors during the warm weather of June.

### Group 2 -- Industrial pollutants

Modelling the indoor levels for sulphur is simpler than for the soil elements since during the summer, the indoor source term is found to be zero for this element. Therefore, the indoor sulphur concentration can be modelled as simply a constant fraction of the outdoor sulphur level. The coefficients $c_1$ and correlation coefficients between the indoor and outdoor scans are given below in table 2, and figure 4 shows the type of fit obtained for S1.

Table 2

| | $c_1$ | corr |
|---|---|---|
| S1 - Business room | 0.86 ± 0.03 | 0.830 |
| S2 - Modelling room | 1.06 ± 0.02 | 0.919 |
| S3 - Main office | 0.55 ± 0.02 | 0.761 |

For all cases, the correlation coefficients are high indicating a strong similarity between the trends in the outdoor sulphur levels and those indoors. The above indoor/outdoor ratio, $c_1$, is found to be much higher than the average of 0.14 for the soil elements. This is a result of the smaller average particle diameter and therefore the poorer ventilation filtration expected for sulphur.

In an attempt to better isolate the source of sulphur emissions, a number of air parcel back trajectories were obtained from Atmospheric Environment Services, Toronto for the times around the high sulphur episode on June 8-9. These trajectories are shown in figure 5. From this survey, it became apparent that the most likely source of the sulphur detected during this period is the industrial area to the south of lake Erie. A scanning electron microscope study confirmed the particulate size distribution on these filters to be in the range of 0.2-0.6µm in diameter. This corresponds to the small particle size expected for a combustion source, and which is necessary for long range transport.

### Group 3 -- Automotive emissions

For this group of elements, again it is found that there is no appreciable periodic indoor source term, so the indoor concentrations of Pb and Br will be modelled according to the outdoor concentrations. For Pb and Br, it is found that the indoor concentrations correlate more strongly with the backyard outdoor levels. This is due to the close proximity of the schoolyard sampler to a source of automotive exhaust. Since this sampler is within 100m of a major traffic intersection, the Pb/Br levels recorded there exhibit unusually large fluctuations. Therefore, the data from the backyard filter was used to model the detected indoor Pb/Br particulate concentrations.

Table 3 shows the values of $c_i$ for Pb, as well as correlation coefficients between the indoor and the backyard Pb levels, and figure 6 shows the fit obtained for the S2 filter location. Note the indoor peak in Pb level occurring on June 5 in figure 6 with no corresponding peak in the outdoor Pb level. For this peak, the indoor Pb level reaches a maximum value of about 1.4 $\mu g/m^3$ off the top of the graph. Since a coincident Br peak was observed inside in a ratio indicative of automotive exhaust, this peak is attributed to an indoor source of automobile emissions. This is likely a result of the doors to the auto shop (which is in the next room) being left open. In performing the linear regression and correlation, this high indoor peak was removed from the data set in order to avoid biasing the indoor/outdoor ratio $c_i$.

Table 3

| | $c_i$ | corr |
|---|---|---|
| S1 - Business room | 0.56 ± 0.03 | 0.796 |
| S2 - Modelling room | 0.63 ± 0.02 | 0.808 |
| S3 - Main office | 0.41 ± 0.02 | 0.728 |

In agreement with the $c_i$ coefficient for sulphur, the modelling room has the lowest filtering efficiency (yielding the highest indoor/outdoor coefficient, $c_i$) of all indoor filtering locations. This chart also shows that the filtering efficiency of the ventilation system is higher for Pb than for sulphur, which implies that the sulphur generally resides on smaller particles than the Pb. This hypothesis has been confirmed by scanning electron micrograph studies.

## Recent developments

The filters collected in this year's Feb-Mar sampling period have so far only been analyzed to the point of obtaining the element scans. As yet, no modelling has been done on this data, so this section of the report will discuss only trends in the data. Indoor/outdoor ratios and activity coefficients have not yet been calculated.

Shown in figure 7 are the element scans for the S3 filter location for the period Feb 20 - March 6/86. Some interesting differences in this data can be observed. Firstly, the dust elements seem to be split into two distinctive groups, with the Si, Fe, Zn, Ti group appearing in high concentrations every weekday, but the Ca, Cl, Cu, S group only appears on certain weekdays. (The elements Ca and Cl do appear in the first group in roughly the same proportions as during the June sampling period, but they appear in the second group in concentrations that are higher by factors of about 3 and 5 respectively.) It is apparent that there is a link between these two groups for at least some days during the week. Why this link was broken on Feb. 21, 25 and March 5 can now only be speculated. Possibly this could be a result of the doors to the office being closed on those days (the office doors are normally left open). Closing the office doors would yield the observed results if the office dust was deficient in Ca, Cl, Cu and S while the dust in the neighbouring hallway was rich in these elements. The high Ca and Cl levels in the hallway could be explained by the large number of people tracking road salt (containing $CaCl_2$) into the hallway from outside, whereas relatively few people would enter the office after just being outside.

It is interesting that during this sampling period the sulphur is found to have a strong indoor source term, while during the June filtering session there was no measurable indoor sulphur coefficient. The difference may be related to the source of Ca and Cl in the hallway. The amount of sulphur in snow due to acid precipitation has been measured to be about 1000 ng/g for sites on Montreal Island.[2]) If the snow in the Kingston area has a comparable sulphur content, then it is possible that the sulphur from snow is deposited on dust particles as the snow melts and evaporates in the hallways of the school. It should be noted, however, that the outdoor sulphur levels still have an appreciable effect on the indoor air. The effect of the outdoor sulphur levels for this period can be seen on March 3-4 when the outdoor sulphur level gradually built up to a peak at about midnight on March 4. Also, the effect of a peak in the outdoor sulphur level occurring in the afternoon of Feb. 23 can be observed in the sulphur scan of figure 7.

Looking at the outdoor element scans for the Feb-March sampling period, the amount of airborne dust is lower on average by a factor of 2-3 than for the corresponding location during the June run. This was expected, and is due to the snow cover which suppresses the injection of dust into the air by a strong wind as was observed in June/85.

## Summary

Using a simple two term model, the indoor particulate concentrations for most elements detected have been adequately explained. For the anthropogenic pollutants, Pb, Br and S, only one term was necessary for the data taken during the June filtering period, and the indoor concentrations for these elements could be described as a constant fraction of the corresponding outdoor concentration. For the naturally occurring elements, a second term was necessary in order to model the daily variations in the concentrations of the elements found in the indoor dust due to human activity within the building. The form of the function used in this term was approximated theoretically, however no allowance for day to day fluctuations in the indoor activities was made. The result was a good prediction of the general trends of the elemental composition of indoor suspended particulate matter for both anthropogenic and naturally occurring elements.

## References

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2) R.E. Jervis and S. Landsberger, **Trace Elements in Wet Atmospheric Deposition,** Intern. J. Environ. Anal. Chem., 1983, Vol 15, pp. 89-106.

3) R.A. Wadden and P.A. Scheff, Indoor Air Pollution, Wiley, 1983.

4) The Case Against the Rain, Ontario Ministry of the Environment report, 1980.

5) V. Valkovic, Trace Element Analysis, London, 1975.

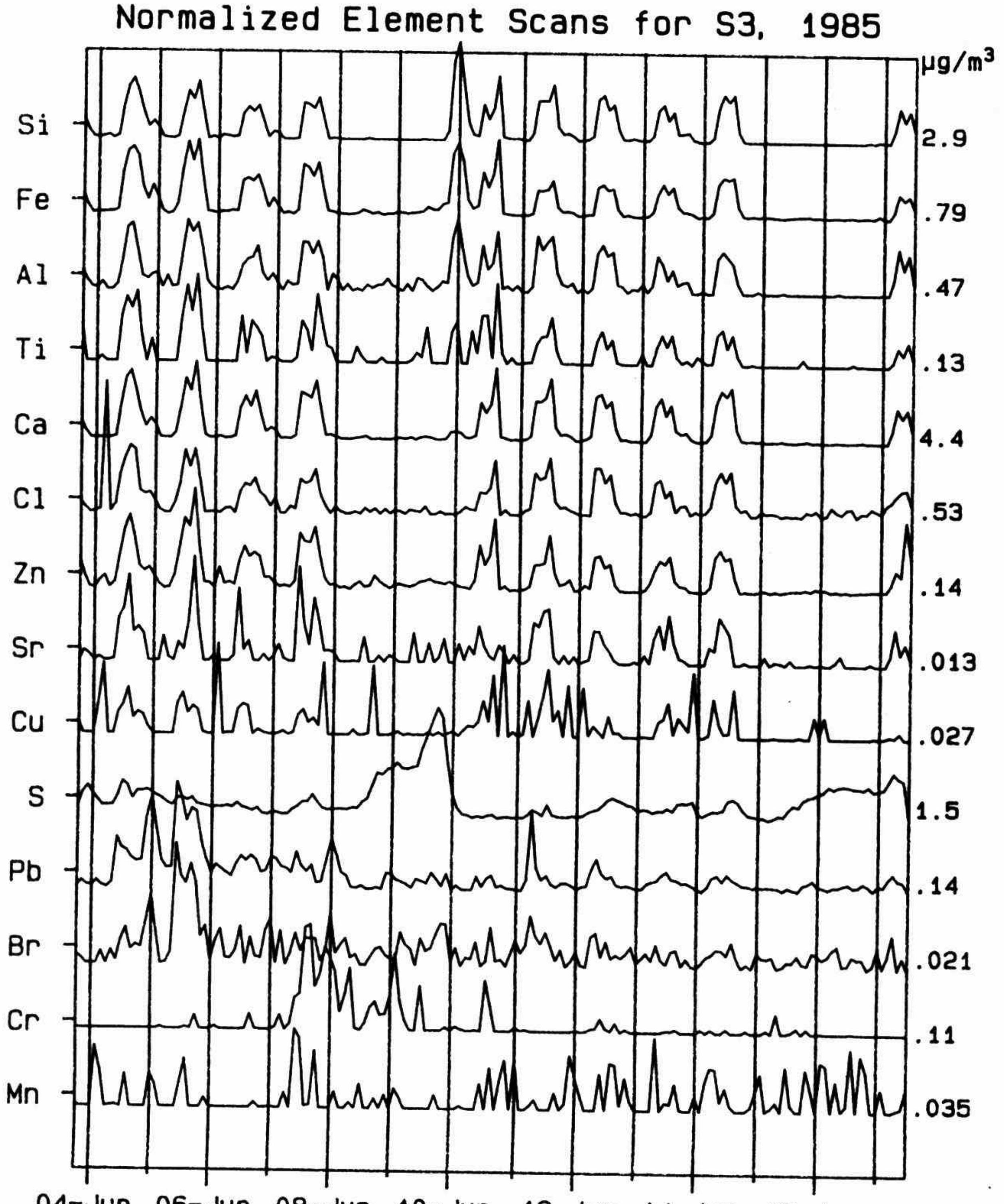

Figure 1 - Indoor element scans for the S3 (main office) filter location for the June/85 sampling period. All scans have been normalized to the same standard deviation, and have been separated by 4 standard deviation units. This separation is given on the right in µg/m³ for each scan.

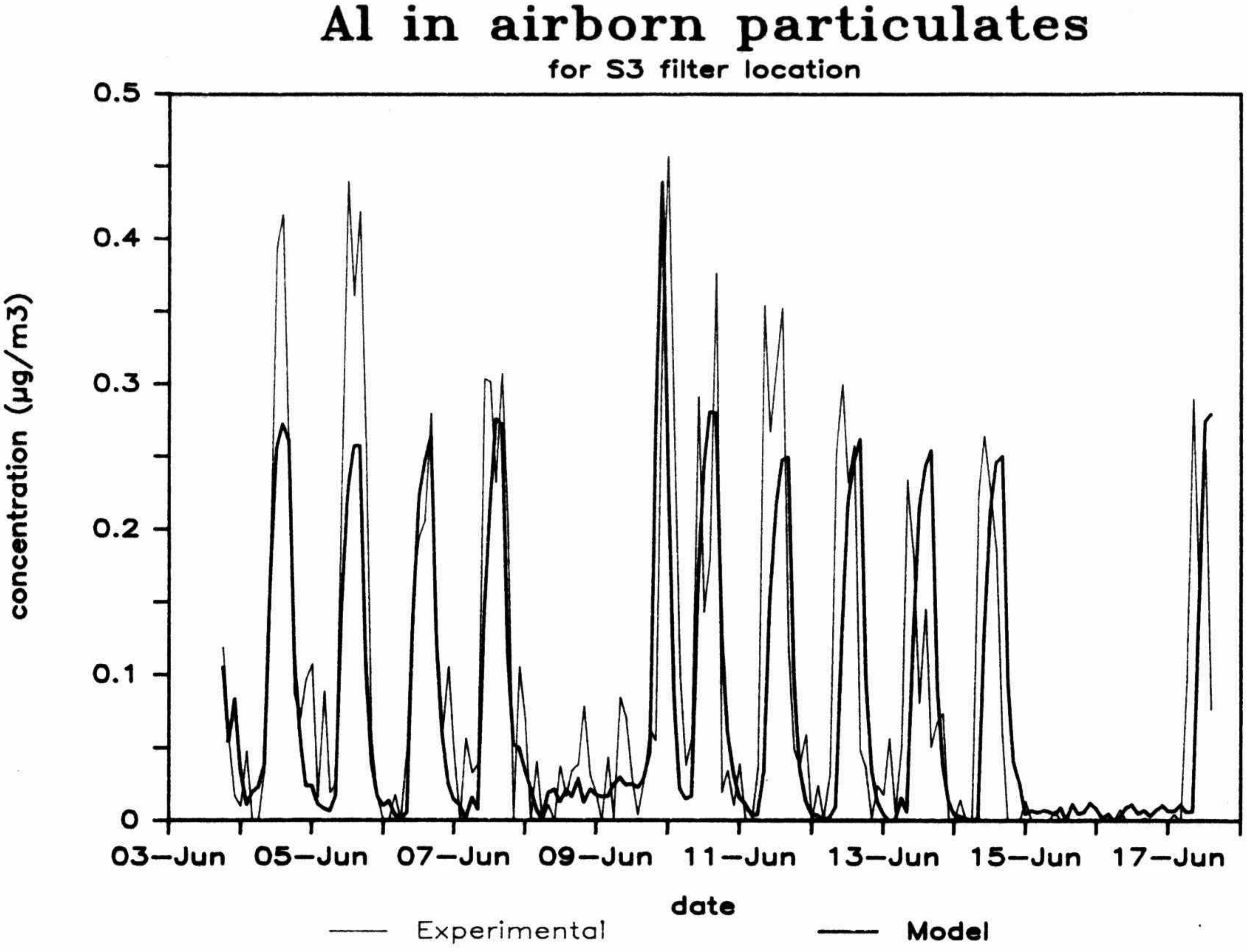
Al in airborn particulates
for S3 filter location
concentration (µg/m3)
0.5
0.4
0.3
0.2
0.1
0
03–Jun
05–Jun
07–Jun
09–Jun
11–Jun
13–Jun
15–Jun
17–Jun
date
Experimental
Model

**Figure 3**

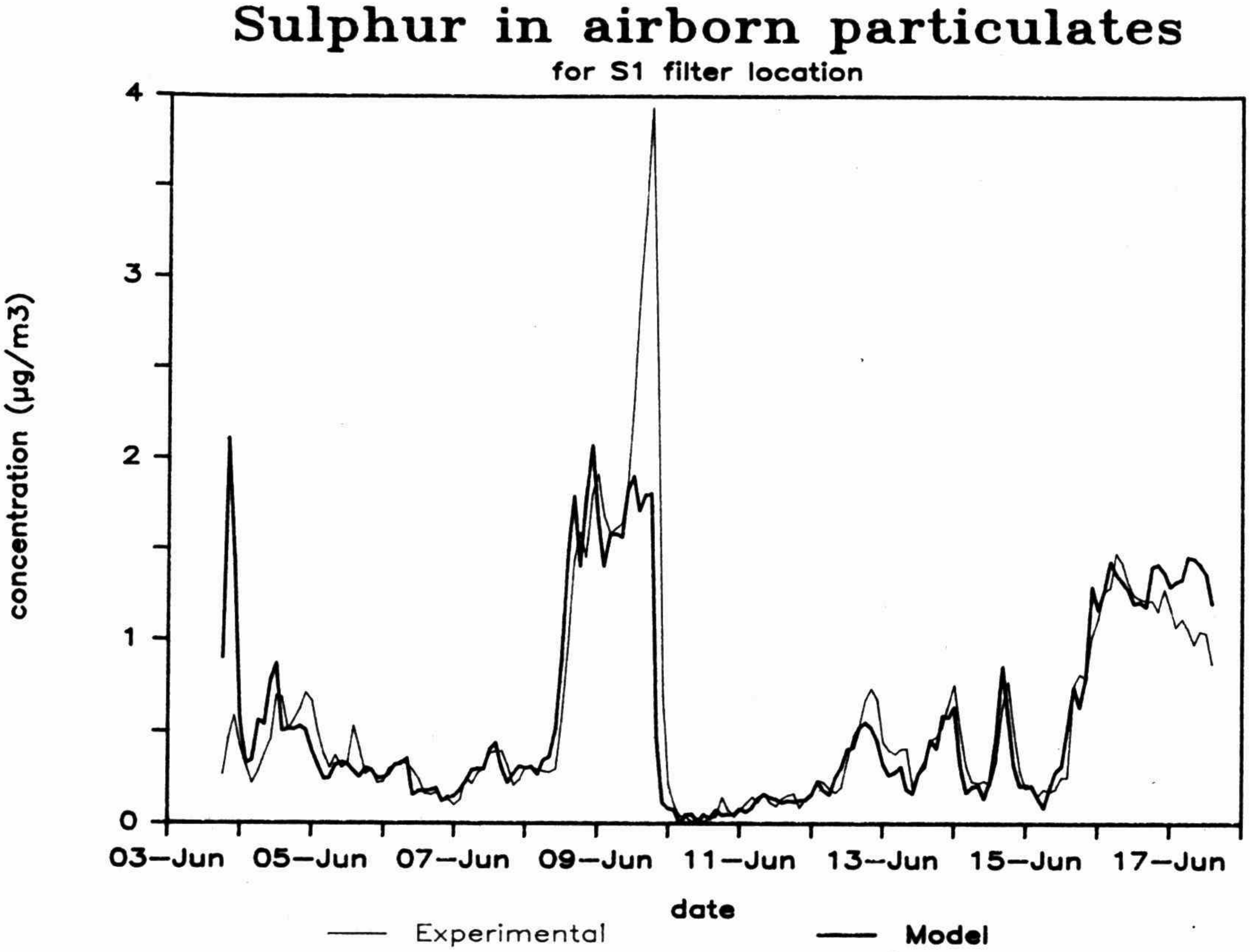
Sulphur in airborn particulates
for S1 filter location
concentration (µg/m3)
0
1
2
3
4
03–Jun
05–Jun
07–Jun
09–Jun
11–Jun
13–Jun
15–Jun
17–Jun
date
Experimental
Model

Figure 4

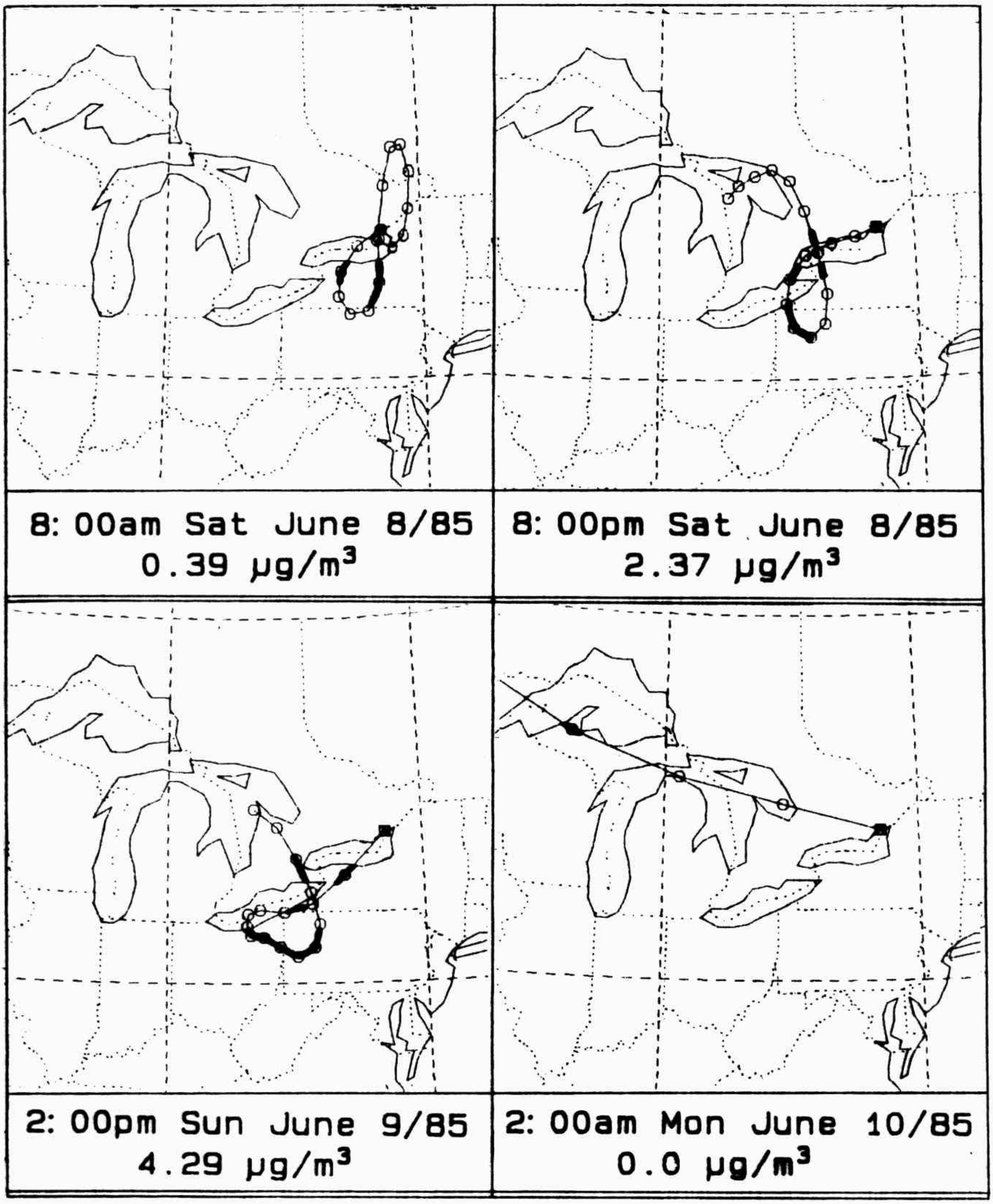

Figure 5 - Four day air parcel back trajectories for the 925mb pressure level. Darkened areas along trajectory paths indicate when the airmass passed over areas of industrial activity. Sulphur levels recorded in Kingston are given in µg/m³ for each trajectory.

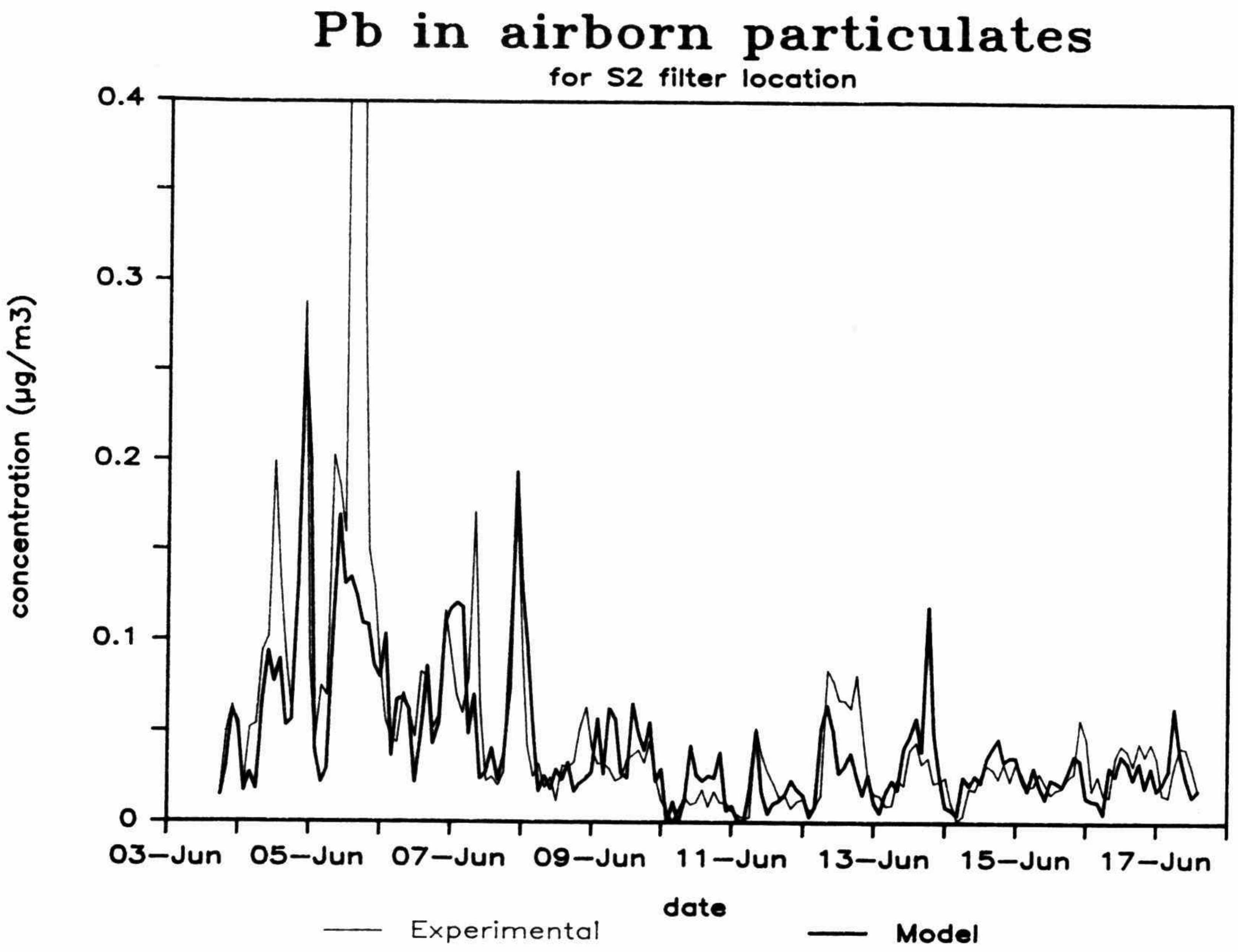
Pb in airborn particulates
for S2 filter location
concentration (µg/m3)
0.4
0.3
0.2
0.1
0
03–Jun
05–Jun
07–Jun
09–Jun
11–Jun
13–Jun
15–Jun
17–Jun
date
Experimental
Model

**Figure 6**

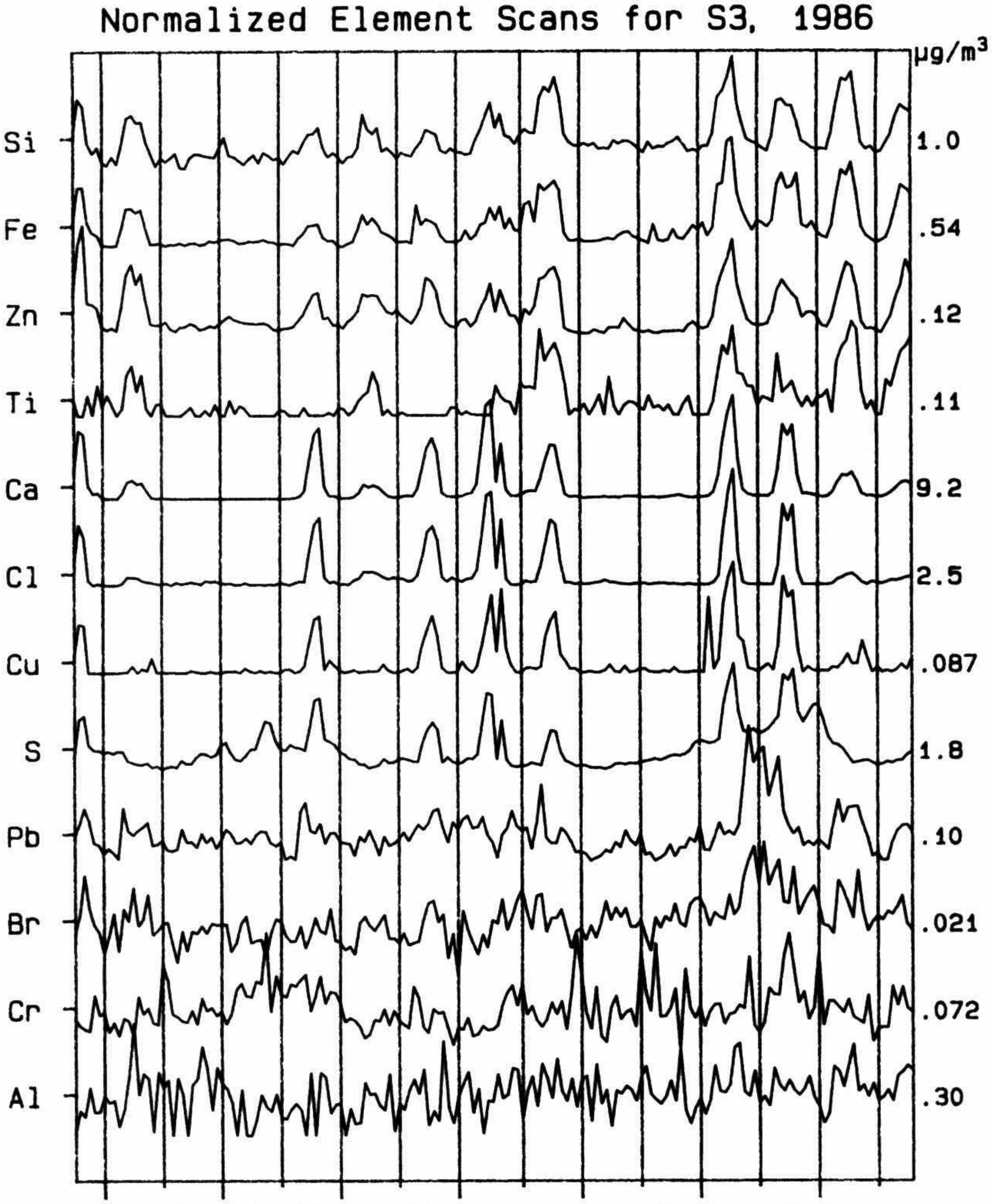

Figure 7 - Indoor element scans for the S3 (main office) filter location for the period Feb. 20 to March 6, 1986. All scales have been normalized to the same standard deviation, with the numbers to the right giving the separation between scans in terms of the individual element concentrations in μg/m³

# STUDY OF THE THERMAL REACTIONS OF POLYCHLORINATED DIBENZO-P-DIOXINS ON FLYASH PARTICLES UNDER INCINERATOR CONDITIONS

by

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## SUMMARY

All incinerators burning municipal waste produce chlorinated dioxins. The mechanism by which these compounds are formed is unknown. Experiments were performed that show these compounds can be produced from known precursors by surface catalysed reactions on the flyash particulates present in the incineration process. A full range of the tetra- through octachlorinated dibenzo-p-dioxins were produced from $^{13}C$-labelled pentachlorophenol and two trichlorophenols on the surface of flyash from an Ontario municipal incinerator that had been previously freed from organic material. A simple flow tube apparatus at 300°C under a 10mL/min. nitrogen flow was used for the experiments. The use of $^{13}C$-labelled pentachlorophenol gave a direct measure of the extent of the catalytic reactivity of the flyash surface. Much lower amounts of chlorinated dioxins were produced in similar experiments with flyash from a modern Japanese incinerator whose effluents are normally extremely low in these compounds. Only small amounts of octachlorodioxin, the thermal condensation product expected, were formed using ground firebrick or an empty flowtube for the reactive surface. These results indicate that the flyash surface has constituents and properties that promote the production of chlorinated dioxins from chlorinated phenols and support the catalyzed surface reaction mechanism previously proposed.

## INTRODUCTION

The incineration of municipal refuse has become an attractive alternative to the practice of dumping refuse in landfill sites. Incinerating refuse in plants close to urban areas offers the advantages of low transportation costs, reduction in the volume of solid wastes and the possibility of energy recovery. However, the incineration of refuse and combustion processes in general emit potentially hazardous substances into the environment. In a review of organic compounds found in incinerator effluent, Junk and Ford listed over 330 substances, including aliphatics, polynuclear aromatic hydrocarbons, substituted benzenes, carbonyl compounds and sulphur and nitrogen-containing compounds (1).

Since 1977 when Olie discovered chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans in the effluent of a municipal incinerator in the Netherlands, attention has been focused on these toxic compounds (2). Chlorinated dioxins have since been found in flyash from incinerators located in Canada, Japan, Switzerland, France, Norway and the United States (3-7). These compounds are of considerable public concern because certain isomers (e.g. 2,3,7,8-tetrachloro-dibenzo-p-dioxin) are extremely toxic and can cause liver damage and chloracne (8).

Discussions of thermal formation of dioxins have generally dealt with gas-phase reactions and extrapolation from the liquid phase to the gas phase. Three general mechanisms have been proposed:

1. survival of trace levels in the fuel,

2. generation from precursors such as chlorinated phenols,

3. *de novo* synthesis from chemically dissimilar compounds and sources of chlorine (9).

However, reports by Shaub and Tsang have made strong arguments in favour of heterogeneous gas-solid phase reactions as the source of chlorinated dioxins from incineration (10-12). Computer simulations based on models of homogeneous gas phase mechanisms predict lower levels of chlorinated dioxins and chlorophenates than are found in incinerators (11). Therefore heterogeneous gas-solid reactions which have much more favourable kinetics than radical reactions at lower temperatures must be considered. Flyash has many Lewis and Bronsted acid and base sites. A chemisorbed precursor molecule could become very reactive, due to the removal of H or Cl from the bound molecule by fast abstraction by gas-phase radicals or by neighbouring reactive sites on the flyash (12). The activation of the phenol by adsorption, possibly through the hydroxyl group, could be similar to that seen by Sanderman and coworkers, who reported that the pyrolysis of the sodium salt of pentachlorophenol gave much larger yields of octachlorodioxin than the pyrolysis of pentachlorophenol alone (13). Experiments using chlorinated dioxins adsorbed onto flyash have shown that gas-solid phase chlorination of dioxins is rapid and efficient (14-17).

In this study chlorinated phenols were passed through a bed of municipal incinerator flyash at 300°C. Tetrachloro- through octachlorodioxin reaction products were detected in the flyash extract and downstream impinger.

## EXPERIMENTAL

Chemicals and Standards: Pentachlorophenol was purchased from Aldrich Chemical (Milwaukee, Wis.). 2,4,5-trichlorophenol and 3,4,5-trichlorophenol were purchased from Fluka (Caledon Labs, Georgetown, Ont.). Unlabelled chlorinated dibenzo-p-dioxin standards were supplied by the Ontario Ministry of the Environment. $^{13}C_{12}$-labelled tetrachlorodioxin and octachlorodioxin were purchased from Cambridge Isotopes Labs (Woburn, Mass.) and labelled pentachlorophenol from MSD Isotopes (Dorval, P.Q.). All chemicals were 98% pure or better. All solvents were of "Distilled in Glass" grade (Caledon Labs, Georgetown, Ont.). Chromosorb P Non-Acid Washed chromatographic support derived from crushed firebrick was purchased from Johns-Mansville (Denver, Colo.).

Flyash: Flyash from a refractory-wall mass-burning municipal refuse incinerator in Ontario was supplied by the Ontario Ministry of the Environment. Machida flyash from a modern fluidized-bed municipal refuse incinerator in Japan was supplied by Dr. H. Hatano, Kyoto University, Kyoto, Japan.

Apparatus and Glassware: The apparatus is shown in Figure 1. All glassware was made of Pyrex.

Glassware was washed in an ultrasonic bath for approximately thirty minutes using a mild detergent. Following rinsing with deionized water, glassware was dried overnight in an oven at 240°C. Before use glassware was rinsed with solvent. Soxhlet extraction apparatus was rinsed by refluxing benzene for two hours. The rinse benzene was discarded and fresh benzene used for the flyash extraction. This procedure has been shown effective in removing background impurities from laboratory glassware.

Experimental Procedure: About 20g of exhaustively extracted flyash was placed in the reservoir. This flyash had been heated overnight in the flowtube at 340°C under a 150 mL/min. flow of nitrogen to remove any residual organic material. A bed of glass beads about 35mm in depth was placed in a section of pyrex tube which had a coarse glass frit in one end. The glass beads were retained with a plug of glass wool. 100uL of a 1 mg/mL chlorinated phenol solution in methanol was deposited onto the glass beads and the solvent allowed to evapourate. The tube was fitted to the reservoir with the frit and glass beads just upstream of the flyash. Attached to the other end of the reservoir was a long-armed impinger of 200 mL capacity. This impinger is similar in design to the one used in the Ames Vapour Sampling System (18). The apparatus was placed in a modified gas chromatographic oven. The impinger resided outside the oven and was cooled with a saturated salt-water-ice bath. A guard trap containing florisil was attached to the outlet of the impinger. The oven was heated to 300°C over twenty minutes then a 10 mL/min. flow of high-purity nitrogen was passed through the flow tube for one hour, maintaining the 300°C temperature throughout. After the experiment was complete the nitrogen flow was stopped and the oven quickly cooled to room temperature. The impinger was removed and the ends sealed with aluminum foil. After rinsing the outside surface of the reservoir with solvent, it was placed in a soxhlet extractor and the organic compounds extracted with 300 mL benzene for 18 hr. The inside surfaces of the impinger were rinsed with 75 mL of dichloromethane, which was transfered to a 300 mL round bottom flask. The extracts were reduced to several mL by rotary evapouration under reduced pressure. In the event that particulate matter was suspended in the extract after rotary evapouration, the extract was transfered to a centrifuge tube and centrifuged to drive the particles to the bottom. The extract was transfered to a pear-shaped flask and concentrated further. Final concentration to 100 uL was achieved by flowing a gentle stream of high purity nitrogen over the surface of the sample in a calibrated 1 mL reactivial.

_Gas Chromatographic Analysis_: To screen the flyash extracts and impinger rinses for chlorinated reaction products samples were analysed on a Hewlett-Packard HP-5880 gas chromatograph equipped with an electron capture detector and a cool on-column injector. A 30m X 0.32mm I.D. DB-5 fused silica capillary column (J & W Scientific, Rancho Cordova, CA, U.S.A.) was used.

_Gas Chromatographic / Mass Spectrometric Analysis_: Analyses were carried out on a Hewlett-Packard 5987A gas chromatograph/mass spectrometer (GC/MS) with an HP1000 data system. The instrument was equiped with a cool on-column injector, a fused silica 30m X 0.32mm I.D. DB-5 column and a direct inlet GC/MS interface. Instrument operating parameters are given in Table I. Quantitation of chlorinated dioxins was carried out in electron impact selected ion monitoring (EISIM) mode. Three isotope ions (M, M+2, M+4 or M+2, M+4, M+6 were monitored for tetra to octachloro-p-dioxins.

The standard mixture used for external standard quantitation of dioxins contained one isomer each of tetra, penta, hexa, hepta and octachlorodibenzo-p-dioxins. Identification of compounds was confirmed using the following criteria:

1. The mass chromatographic peaks must fall within a retention time window established for a specific class of isomers.
2. The mass chromatographic peaks produced by the component must exhibit the correct ratios of intensities of ions being monitored.
3. The signal-to-noise ratio must be greater than 3.

The retention time windows were determined by using mixtures of available isomeric compounds.

## RESULTS AND DISCUSSION

Labelled $^{13}$C-pentachlorophenol was passed through a flowtube containing either Ontario flyash or Machida flyash at 300°C using a flow of 10mL/min N2 maintained for one hour. The GC-ECD analyses of the flyash extracts are shown in figure 2. The top chromatogram is an analysis of the extract of the Ontario flyash, the second an analysis of the extract of the Machida flyash. The bottom plot is an analysis of a standard solution containing tetrachloro- to octachloro-dioxins and furans at an average concentration of 2 nanograms per microlitre. The peaks in the chromatograms represent reaction products or the unreacted labelled phenol. Compounds eluting below 200 degrees Celcius are mostly chlorinated benzenes and chlorinated phenols. The compounds eluting above this temperature are the result of the condensation of the chlorinated phenol and other molecular reactions which followed. The experiment using the Ontario flyash yielded much larger amounts of some reaction products than that using Machida flyash. These reaction products elute in the retention time windows characteristic of chorinated dioxins and furans.

The chromatograms from the EISIM analyses of the flyash extract and the impinger rinsings from the experiment using Ontario flyash is shown in figure 3. The plots were produced by merging the selected ion abundance profiles (SIAP) of the most abundant ion from the molecular ion isotope cluster of each $^{13}C_{12}$-labelled chlorinated dibenzo-p-dioxin. The use of a labelled precursor eliminates interferences from possible unextracted compounds on the flyash. It is clear that chlorinated dioxins were formed in-situ on the surface of the flyash from the pentachlorophenol precursor. Octachlorodibenzo-p-dioxin is likely formed first by the condensation of two phenol molecules. The other chlorinated dioxins arise through the dechlorination of the higher chlorinated species.

The chlorinated dioxins appearing in the impinger rinsings are mainly the tetra- and pentachlorinated isomers. These more volatile compounds are desorbed from the flyash and are carried downstream by the nitrogen flow to condense in the cooled impingers. It has been recently demonstrated that chlorinated dioxins will desorb from flyash surfaces under similar conditions of temperature and flow (19).

The results of the EISIM analyses of the flyash extracts and impinger rinsings from the experiment using Machida flyash is shown in figure 4. The chlorinated dioxin elution pattern in the flyash extract is remarkably similar to that of the Ontario flyash. The peak at 26.5 minutes in the analyses of the flyash and the impinger rinsings is an impurity, likely a phthalate ester.

The amounts of chlorinated dioxins formed from labelled pentachlorophenol are shown in figure 5. The experiment using Ontario flyash produced almost ten times as much chlorinated dioxins as the Machida flyash. It would appear that the latter flyash has a lower catalytic activity than the Ontario flyash. This could be due to differences in the composition of the incinerator fuel feeds or differences in the design and operating conditions of the incinerators.

Both Ontario and Machida flyashes provide a large surface area on which reactions could take place. To determine whether a large surface area is sufficient to produce the large number of isomers observed from reactions of pentachlorophenol, the precursor was passed through a bed of ground firebrick and through an empty flowtube. The results of these experiments are shown in figure 6. The chromatograms are from EISIM analyses of the impinger rinsings from experiments using Ontario flyash, firebrick and an empty flowtube. The Ontario flyash trace shows a large amount of chlorinated dioxins being formed as compared to the empty flowtube or the firebrick experiments. In the latter two experiments, the majority of the chlorinated dioxins were in the form of octachlorodibenzo-p-dioxin, the least toxic isomer of the chlorinated dioxins. The experiment with the Ontario flyash produced tetra- through octachlorodibenzo-p-dioxins.

It is clear that surface area is not a factor in producing large numbers of dioxin isomers from pentachlorophenol. The ground firebrick has a specific surface of 3.8 $m^2/g$, which is comparable to the Ontario flyash specific surface area of 3.2 $m^2/g$ and the Machida flyash specific surface area of 2.2 $m^2/g$ . These surface areas are orders of magnitude smaller than those of heterogeneous catalysts. These results indicate that the flyash surface has constituents and properties that promote the production of chlorinated dioxins from pentachlorophenol.

The total amounts of chlorinated dioxins formed using flyash, firebrick and the empty flowtube are presented in figure 7 for comparison. Octachlorodibenzo-p-dioxin and heptachlorodibenzo-p-dioxins constitute over seventy-five percent of the 3200 nanograms of chlorinated dioxins produced on the flyash. Approximately 40 ng, 100 ng and 400 ng of tetra-, penta- and hexachlorodibenzo-p-dioxins were produced, respectively. Only using an empty flowtube was there a significant amount of octachlorodibenzo-p-dioxin produced, whereas the firebrick alone produced almost immeasureable amounts. Since the amounts of octachlorodibenzo-p-dioxin produced using firebrick and an empty flowtube correspond to that produced purely by thermal condensation, clearly the catalytic constituents of the flyash promote the condensation of the chlorinated phenols to octachlorodibenzo-p-dioxin. The total amount of chlorinated dioxins produced on flyash was ten times the amount produced by the experiment using an empty flowtube. It was expected that the firebrick and empty flowtube experiments would have produced similar amounts of octachlorodioxin. The difference, we speculate, may be due to the firebrick inhibiting the condensation of the chlorinated phenol.

Having established that Ontario flyash will catalyse the production of chlorinated dioxins from pentachlorophenol, the experiments were repeated using 2,4,5-trichlorophenol and 3,4,5-trichlorophenol. The results of these experiments are shown in figure 8. These chromatograms are made from EISIM analyses of the impinger rinses. The distributions of the dioxin isomer from these chromatograms are clearly very different. The experiments using 2,4,5-trichlorophenol produced mainly hexachlorodibenzo-p-dioxins and very little of the tetra- and octa-chlorinated isomers. Also, within each congener group there are several peaks whose relative abundances are greater than those found in the experiments using pentachlorophenol or in the analyses of chlorinated dioxins extracted from the Ontario flyash before treatment. This would indicate that there are certain isomers whose production is favoured over the others, implying that certain positions on the benzene rings are more reactive to chlorination or dechlorination than the others.

The experiment using 3,4,5-trichlorophenol yielded very interesting results, in that dioxins were formed at all from a chlorinated phenol that did not possess a chlorine atom ortho to the hydroxyl group. Condensation and ring closure to form dioxins from chlorinated phenols requires ortho-substitution. Some scrambling of the chlorine and hydroxyl group positions must take place for dioxins to be formed from this precursor. The dioxin isomer distribution is very different from the distributions arising from 2,4,5-trichloro- and pentachlorophenol. The major peaks are octachlorodibenzo-p-dioxin and 1,2,3,4,5,6,7,8-heptachlorodibenzo-p-dioxin. The relatively small amount of the other heptachloro isomer suggests that the 9-position on the dioxin molecule is less easily chlorinated that the 8-position. The lesser chlorinated isomers were present only at very low levels, with pentachlorodibenzo-p-dioxin detected at the detection limit of 100 pg/uL (100 uL total sample volume).

The total amounts of each dioxin congener group produced in the experiments using penta-, 2,4,5-tri- and 3,4,5-trichlorophenol are shown in figure 9. The major reaction products from the 2,4,5-trichlorophenol isomer are hexa- and heptachlorodibenzo-p-dioxins. Considering that chlorination and dechlorination are competing processes, it would be expected that these dioxins possessing a degree of chlorination midway between the tetrachlorodibenzo-p-dioxins - the condensation product - and octachlorodibenzo-p-dioxin would predominate. The large amount of the octachlorinated and heptachlorinated isomers produced using 3,4,5-trichlorophenol and the lack of any significant amounts of pentachlorodibenzo-p-dioxin would indicate a different route to the dioxins than that taken by the other chlorinated phenols possessing an ortho-substituted chlorine. The phenol may undergo rearrangement or isomerization due to dechlorination, dehydrogentation, transchlorination and other similar molecular reactions before condensation to form a chlorinated dioxin. The various competing reactions and the reactivity of the intermediates would be reflected in the amounts and kinds of isomers formed.

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## Table I: Instrument Operating Parameters

Chromatographic Conditions

Fused Silica WCOT column 30m X 0.32mm I.D.

Stationary Phase DB-5, 0.25 um thickness

Helium carrier gas flow 2.5mL/min.

Temperature programs:

GC-ECD: 80°C for 1 min., to 300°C
at 3°C/min., hold for 5 min.

GC-MS-EISIM: 80°C for 1 min., to 220°C at
20°C/min., to 300°C at
2°C/min., hold for 5 min.

Selected Ion Monitoring Mass Spectrometry

| Ions | m/z | Dwell (ms) |
|---|---|---|
| TCDD | 319.9, 321.9, 323.9 | 100 |
| $[^{13}C_{12}]$-TCDD | 267.9, 331.9, 333.9 | 100 |
| $P_5$CDD | 353.9, 355.9, 357.9 | 100 |
| $[^{13}C_{12}]$-$P_5$CDD | 301.9, 365.9, 367.9 | 100 |
| $H_6$CDD | 387.9, 389.9, 391.9 | 100 |
| $[^{13}C_{12}]$-$H_6$CDD | 335.9, 399.9, 401.9 | 100 |
| $H_7$CDD | 421.8, 423.8, 425.8 | 100 |
| $[^{13}C_{12}]$-$H_7$CDD | 369.8, 433.8, 435.8 | 100 |
| OCDD | 455.7, 457.7, 459.7 | 100 |
| $[^{13}C_{12}]$-OCDD | 405.7, 469.7, 471.7 | 100 |

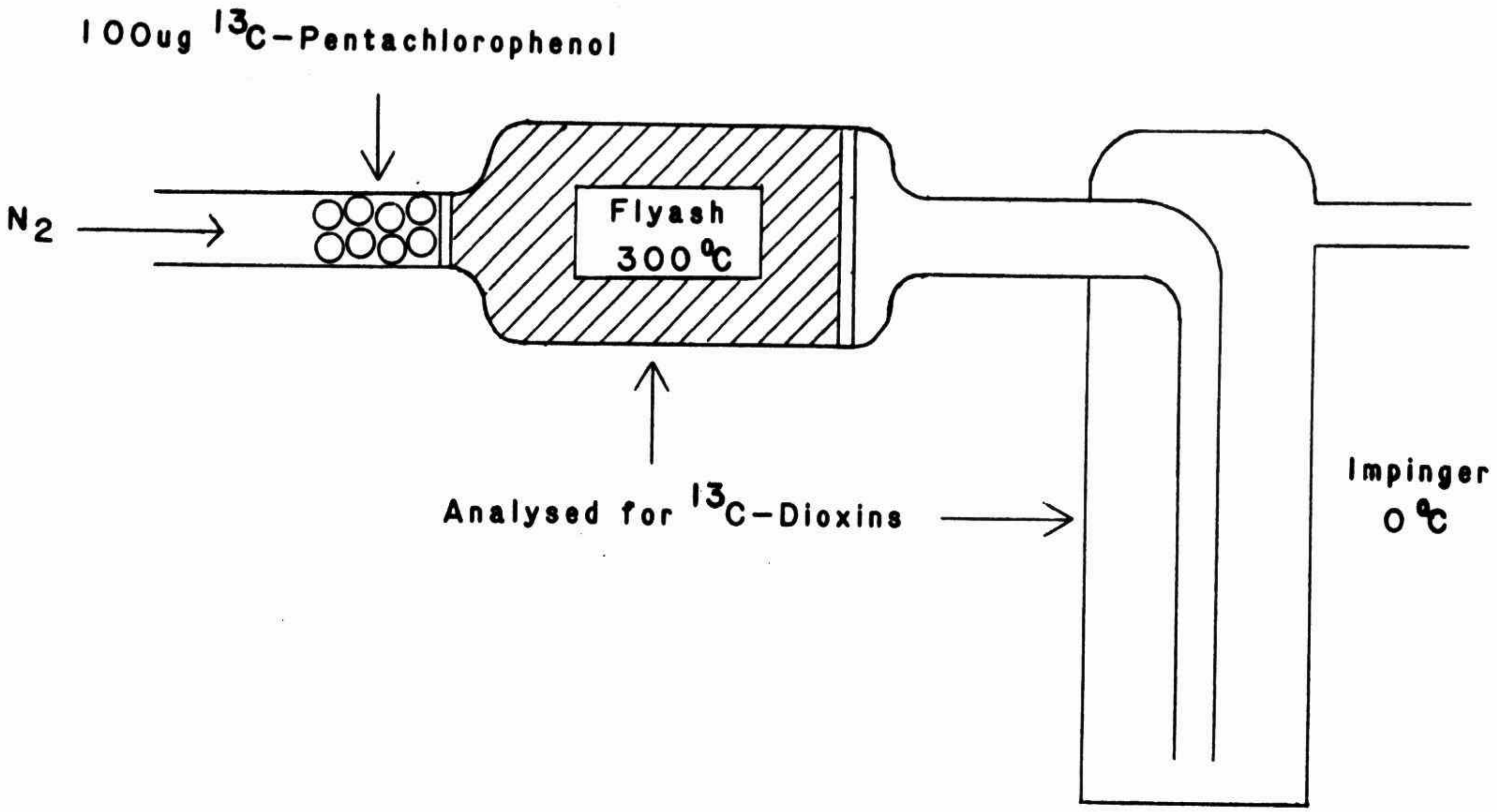
100ug $^{13}$C-Pentachlorophenol
$N_2$
Flyash
300 °C
Analysed for $^{13}$C-Dioxins
Impinger
0 °C

Figure 1: Schematic of Experimental Apparatus. Nitrogen flow carries chlorinated phenols through flyash bed and reaction products are collected downstream in the impinger and extracted from the flyash after the experiment.

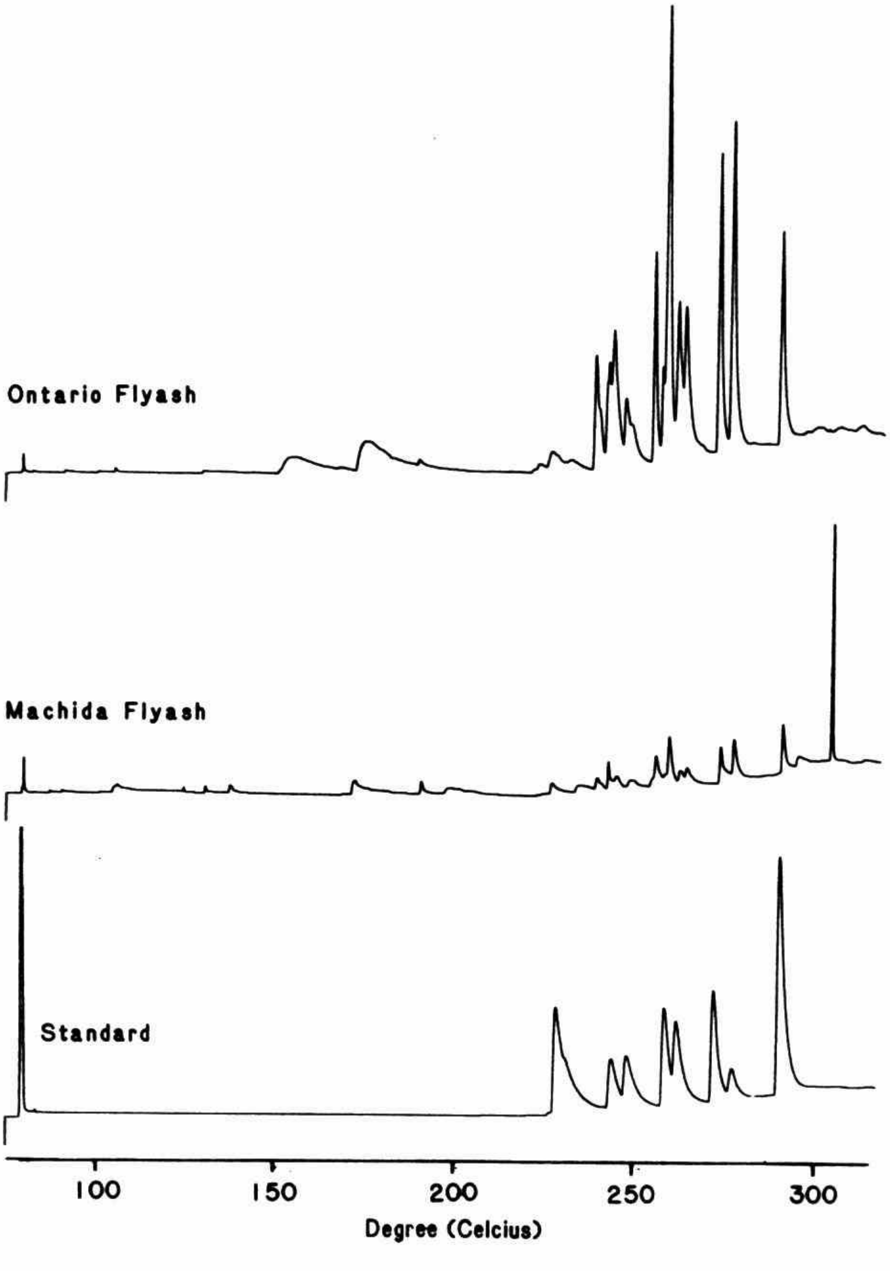

Figure 2: GC-ECD chromatograms of flyash extracts showing reaction products from $^{13}$C-labelled pentachlorophenol.

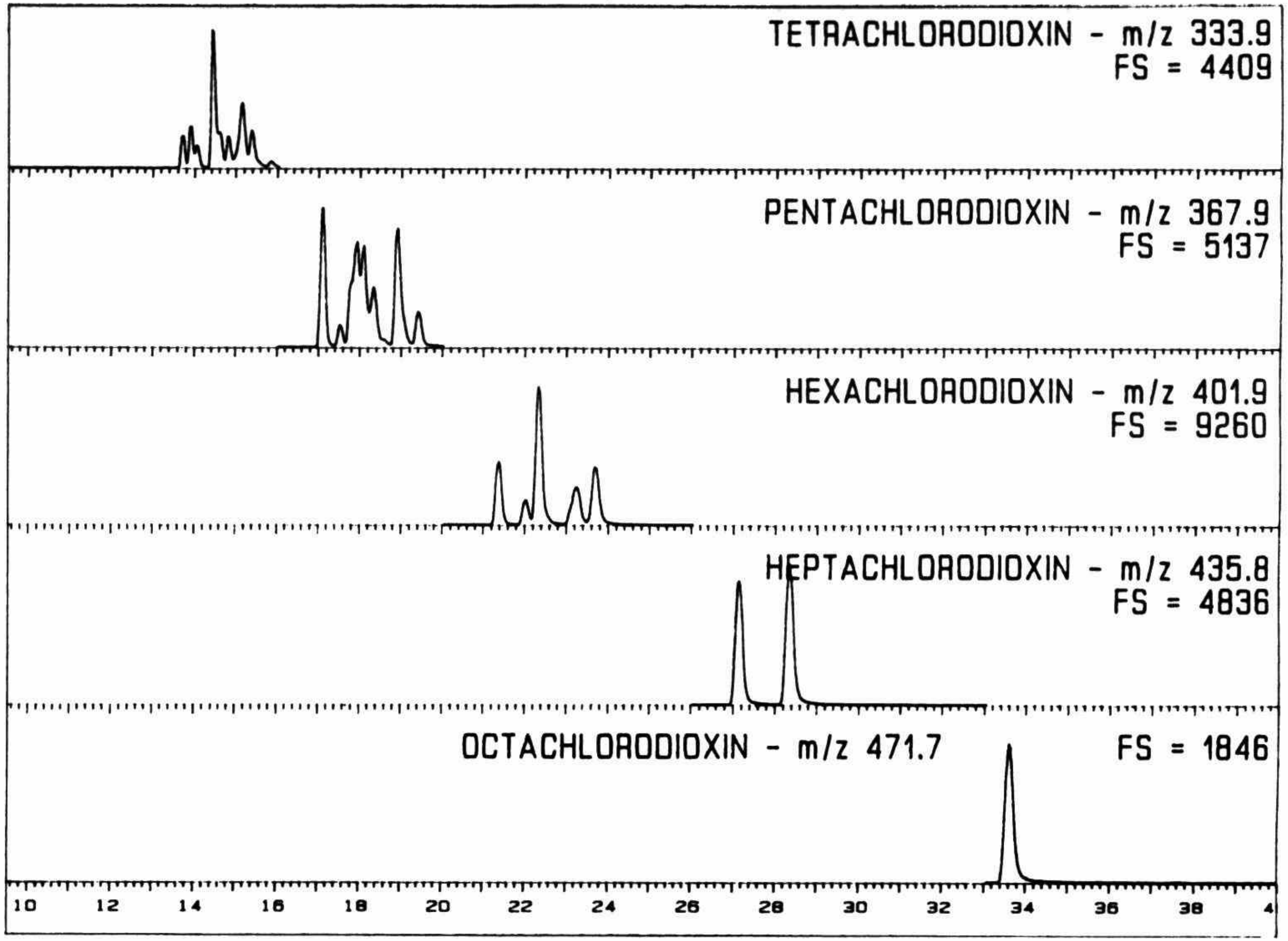

Figure 3: Chlorinated dioxins with $^{13}C$ produced from $^{13}C$-pentachlorophenol using Ontario flyash. Each plot shows the abundance of the base peak of the dioxin congener versus retention time. The ion mass and the plot full scale are indicated at the right of each plot.

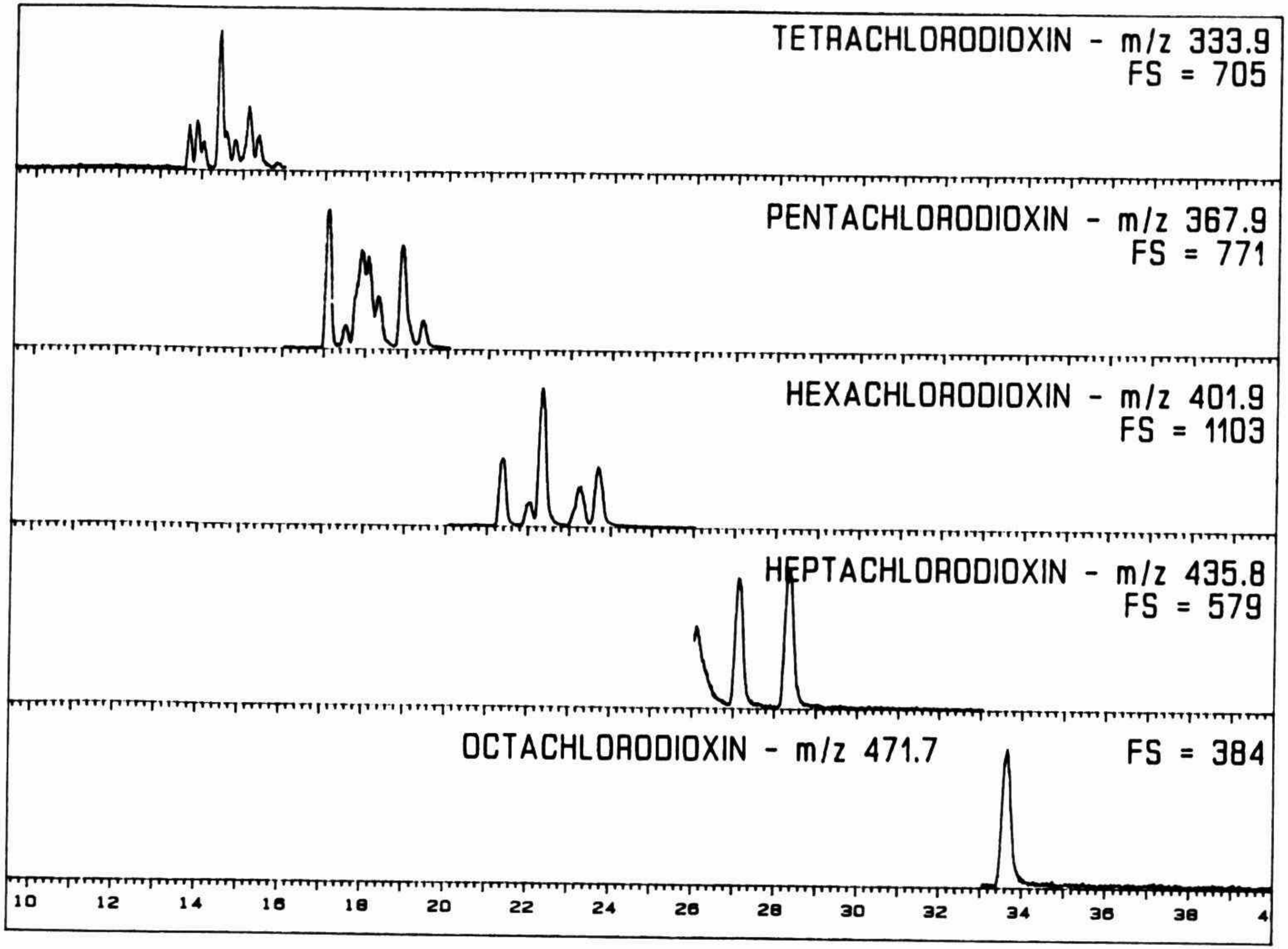

Figure 4: Chlorinated dioxins with $^{13}C$ produced from $^{13}C$-pentachlorophenol using Machida flyash. Each plot shows the abundance of the base peak of the dioxin congener versus retention time. The ion mass and the plot full scale are indicated at the right of each plot.

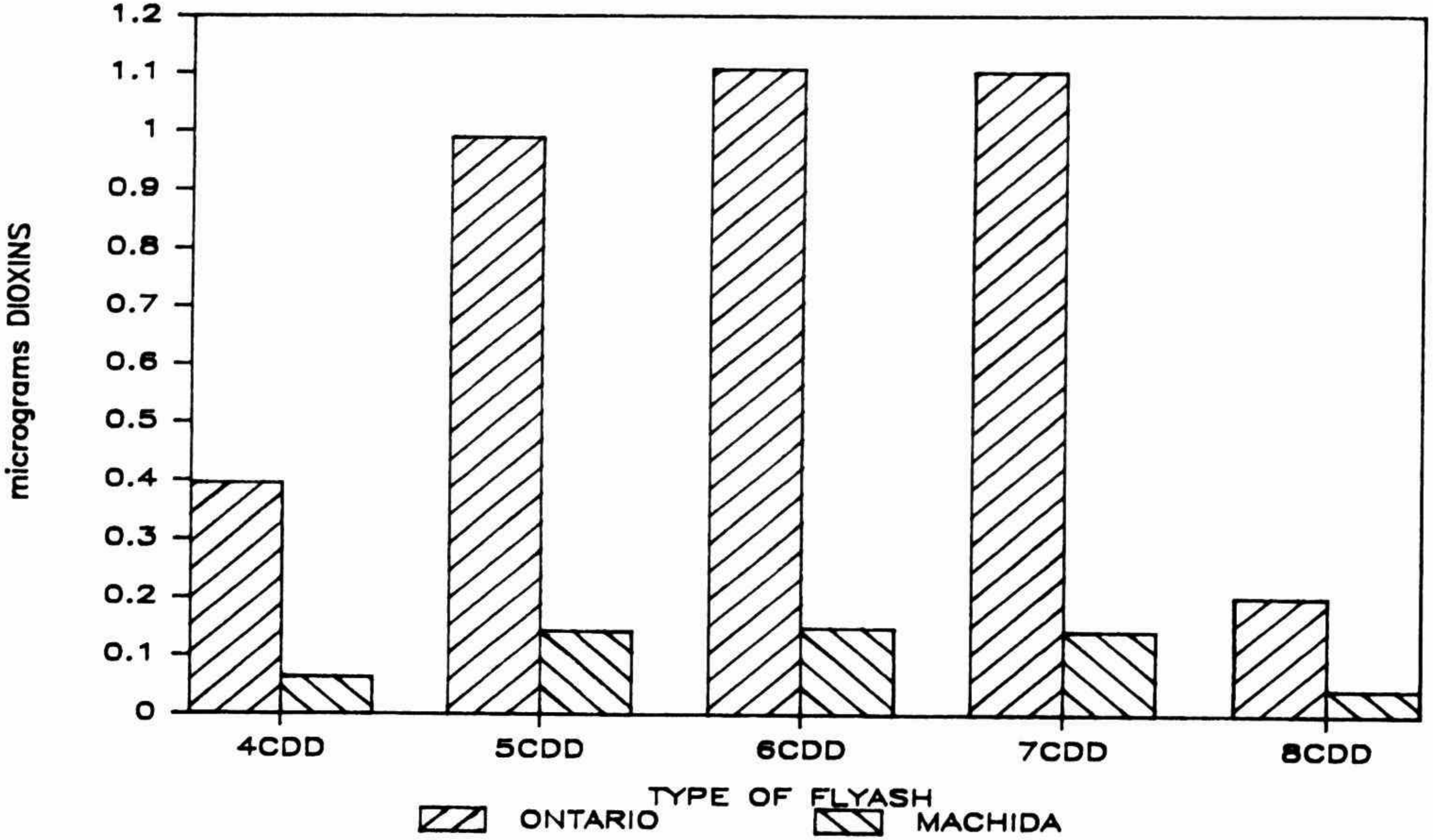

Figure 5: Bar chart showing the amounts of $^{13}$C-labelled chlorinated dioxins formed from 100 micrograms of $^{13}$C-pentachlorophenol using Ontario and Machida flyash. Over 3.2 micrograms of chlorinated dioxins were formed using Ontario flyash.

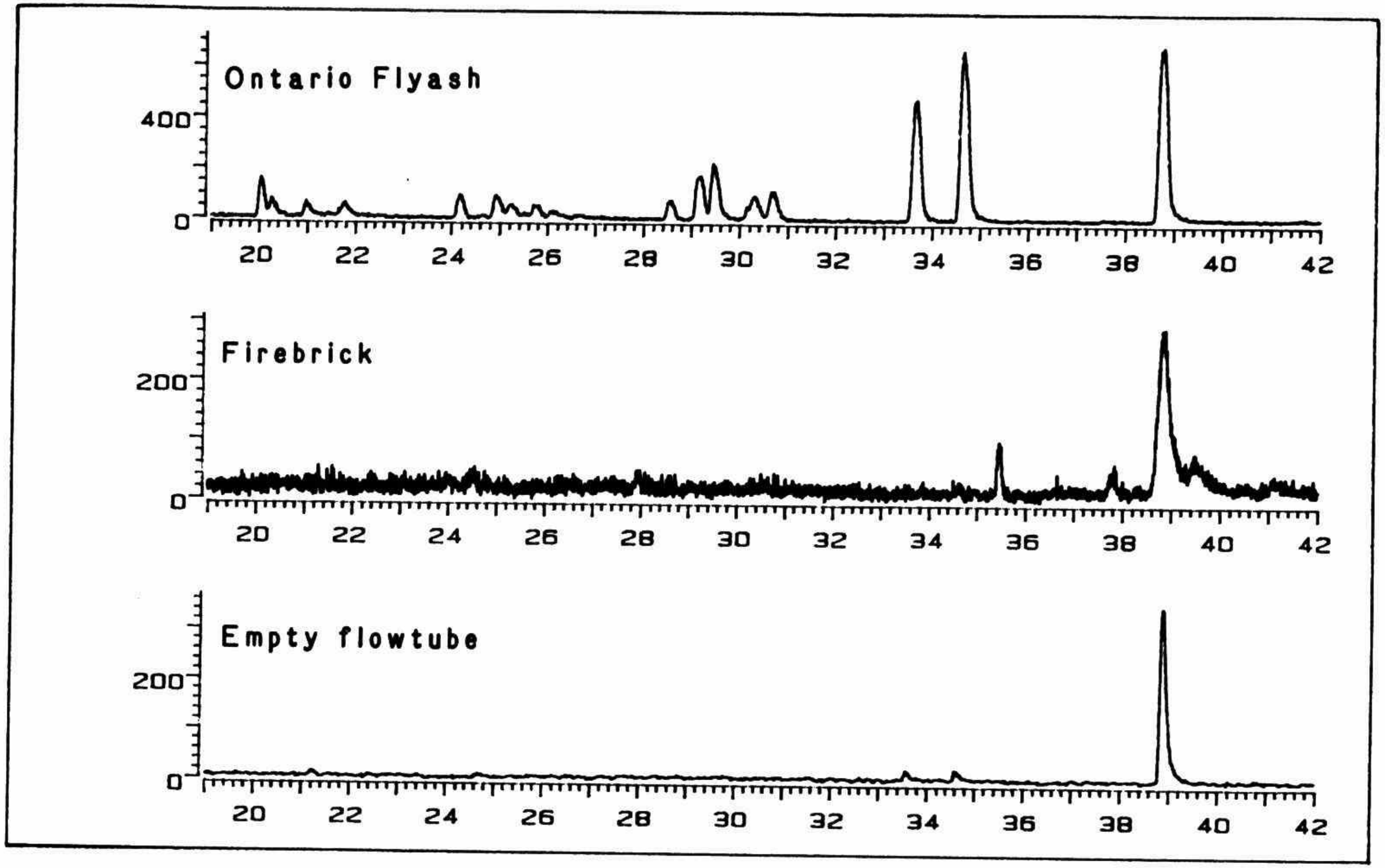

Figure 6: Chlorinated dioxins produced from pentachlorophenol using Ontario flyash, firebrick or an empty flowtube which were found in the downstream impinger rinses.

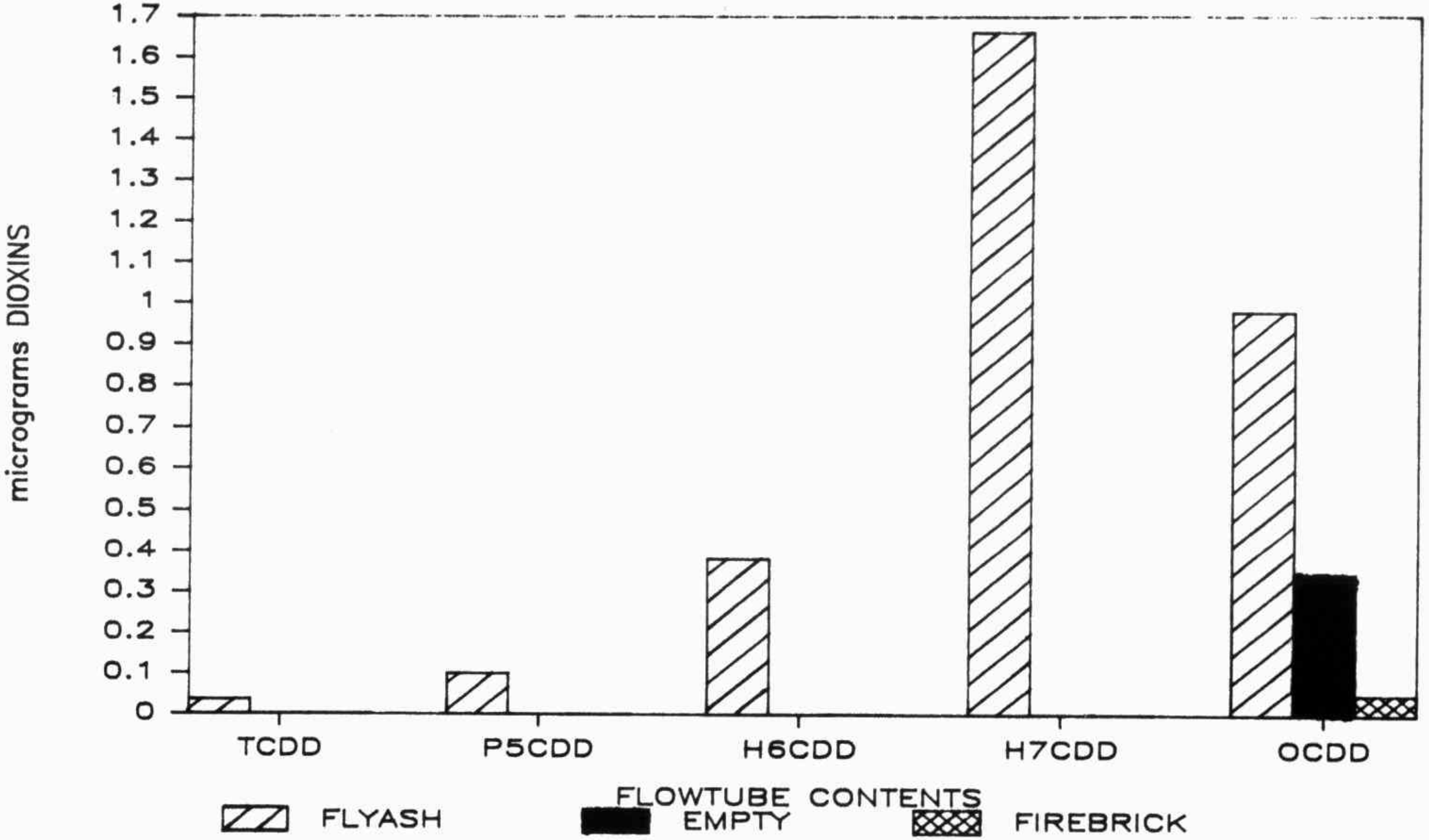

Figure 7: Bar chart comparing the amounts of chlorinated dioxin reaction products formed from 100 micrograms of pentachlorophenol using Ontario flyash, firebrick or an empty flowtube.

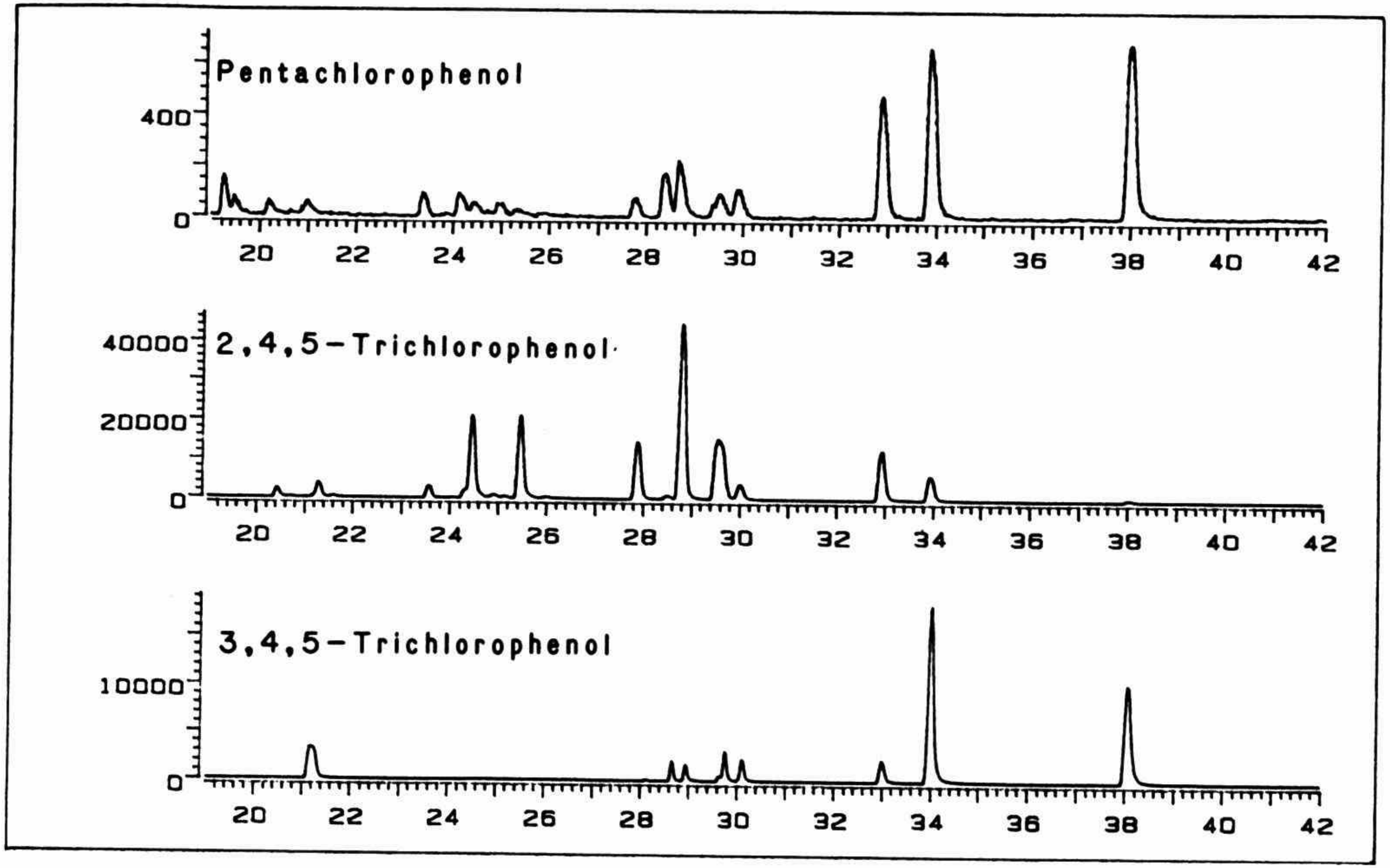

Figure 8: Chlorinated dioxin reaction products formed on Ontario flyash using from three different chlorinated phenols which were found in the downstream impinger rinses. The dioxin isomer distributions appear to be dependent upon the chlorine substitution pattern of the reactant.

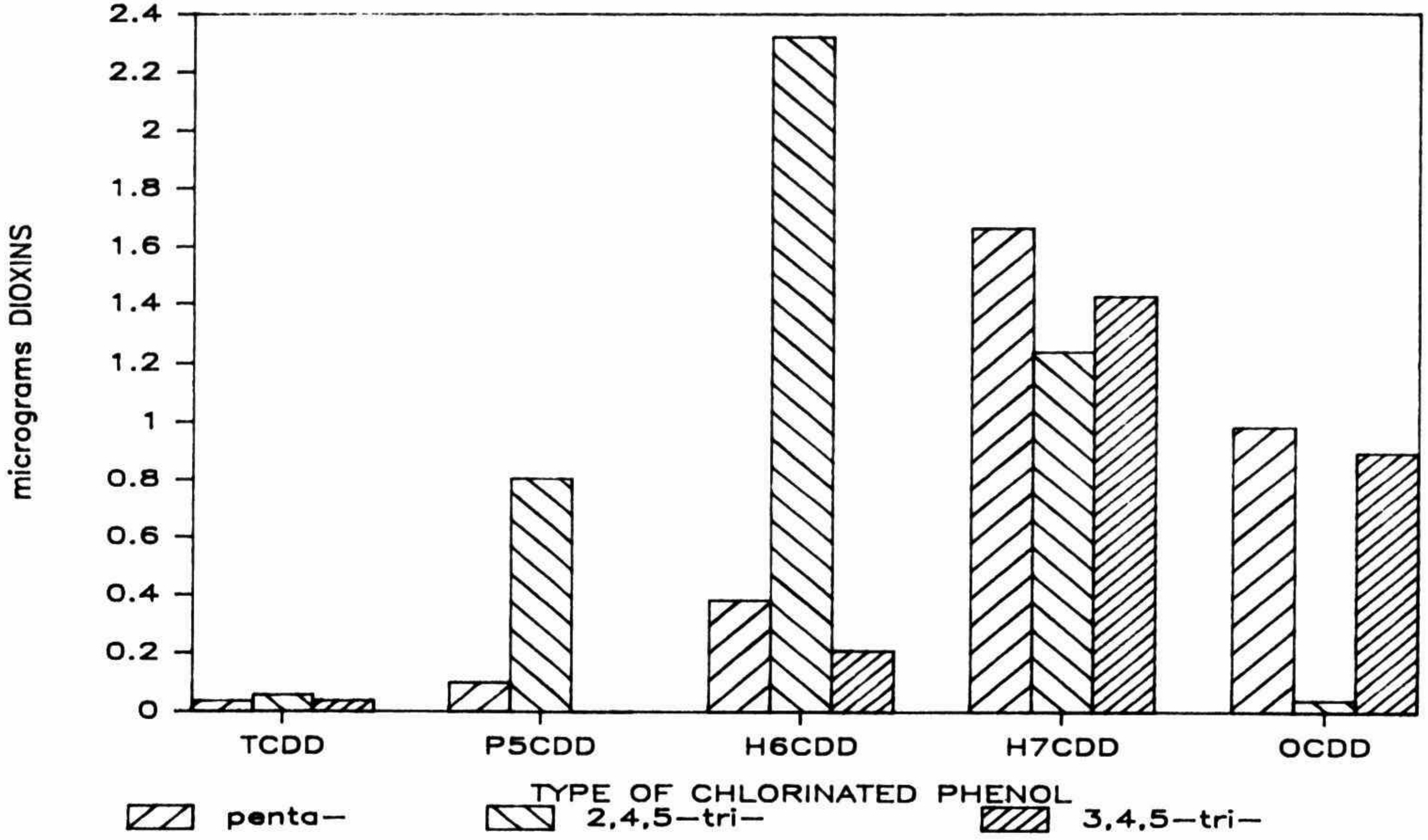

Figure 9: Bar chart comparing the amounts of chlorinated dioxin reaction products formed on Ontario flyash using three different chlorinated phenols.

# TESTING OF A TOTAL HYDROCARBON MONITORING SYSTEM AT THE ASHBRIDGES BAY SEWAGE TREATMENT PLANT INCINERATOR, TORONTO.

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J. Marson, Ministry of the Environment, Toronto

## INTRODUCTION

A continuous emission monitoring (CEM) demonstration at Toronto´s Ashbridges Bay sewage sludge incinerator was carried out. The project consisted of monitoring total hydrocarbon (THC) emissions for a period of 34 days and was carried out by AMKO Systems Inc.

The Ministry of the Environment´s (MOE) Source Assessment Unit (SAU) realized special interest in this project since problems have continued to hamper continuous emission monitoring (CEM) of THC in sludge incinerators. Continuous THC monitoring was mandatory in the Certificate of Approval of the Ashbridges Bay Plant.

The analyzer system installed on site in 1978 had only been functional for a few days. Fundamental design flaws such as insufficiently heated sample-wetted lines and detector in the Horiba analyzer were the cause of this negligible performance. The developments and testing of a quasi maintenance-free monitoring system was found necessary in view of the many problems existing in CEM THC installations.

Plant Management at Ashbridges Bay was contacted and very receptive to our finding a cure for its inoperative THC monitoring system.

The objectives of this study were 1) to design a Flame Ionization Detector (FID) based THC monitoring system, complying with MOE guidelines, that was capable of extended up-time by application of an efficient sample conditioning system and 2) to operate a THC analyzer for a period of 30 days so as to assess CEM up-time, accuracy and maintenance requirements.

## SUMMARY

A total hydrocarbon emission monitoring system was successfully demonstrated at the Ashbridges Bay sewage sludge incinerator stack. The system was at the stack for 34 days and produced valid data for 67% of the time. Most of the down-time was due to chart recorder problems. Had more appropriate funding been made available for daily inspection, 95% up-time would have been realized. During the 546 hours of valid data, the stack gas concentrations averaged 720 ppm, as methane, and the daily averages ranged from 432 ppm to 1229 ppm. These values are in line with previous measurements (1982). The cause of the high THC concentration has not been determined in this work.

## PROCESS DESCRIPTION

Our tests were conducted at the main stack of the Ashbridges Bay sewage treatment plant. The stack vents a number of sludge incinerators with combined throughput of an estimated 13.5 tons of dry solids per hour. The stack gas flow is approximately 32m3/sec. During the program there were typically four units in operation. The incinerators are multiple hearth type and combustion is supported with natural gas fuel. The exhaust gases from each incinerator are cleaned in separate water scrubbers before entering the common stack. The approximate composition of the stack gases is as follows:

| | |
|---|---|
| CO2 | : 2.6% |
| O2 | : 15.0% |
| CO | : 0.5% |
| N2 | : 81.9% |
| Moisture | : 6.5% |
| Particulate | : 10 mg/m3 |
| Temperature | : 33 degress C |

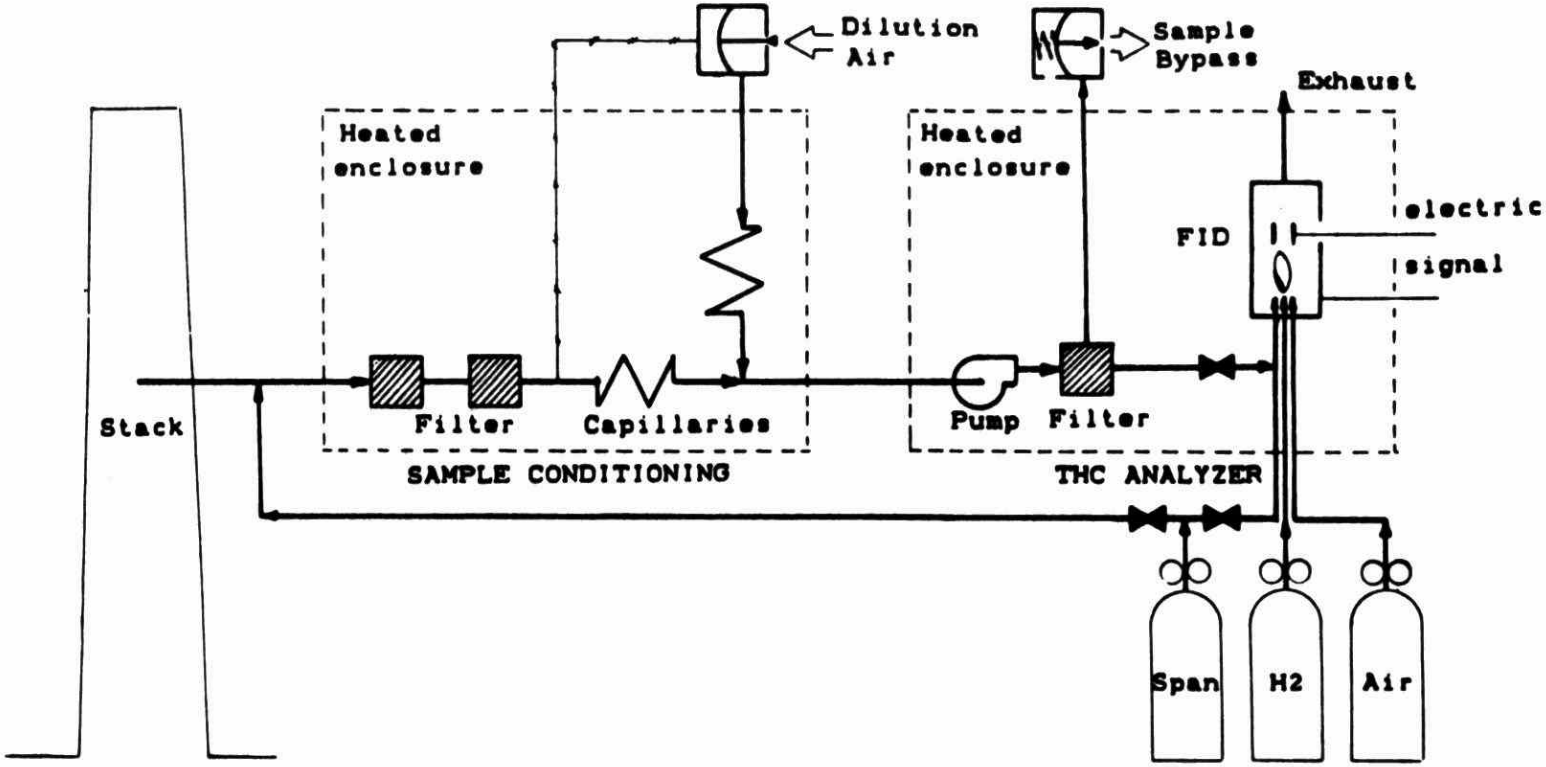
The sampling location is at the 64m elevation of the 183m main stack. The sampling platform is enclosed between the stack liner and the concrete shell, accessible by elevator.

## OUTLINE OF THE MONITORING SYSTEM

The THC monitoring system consisted of a probe, a sample conditioning unit and a THC analyzer. The monitor´s fuel, fuel-air and span gas cylinders were housed in a ground cabinet, adjacent to the stack, and connected to the analyzer via one-quarter inch stainless steel tube. The probe was three meters long and made of one-half inch stainless steel pipe. Our enclosed sample conditioning unit consisted of two filters followed by a dilution system. The analyzer was a Ratfisch RS5 (1974) which is equipped with an FID. The THC monitoring system is depicted schematically in Figure 1.

The stack gas sample was extracted at the rate of about one liter per minute. Particulate matter was removed by coarse and fine filters. The filtering elements were 1" x 2 1/2 " Balston cylinders with efficiency for 0.1 micron particles rated at 93% and 99.99%, respectively. Sample conditioning included a 1.1 sample dilution stage. Dilution was accomplished by throttling the sample flow (1l/min.) with a 30 cm long, 1/8 inch OD tubing coil. A similar coil was used to throttle the flow of dilution air (1l/min.). The inlet pressures of the two capillaries were matched by a modified pressure regulator. The filters and capillaries were maintained at 150 degress C.

FIGURE 1
THC SYSTEM SCHEMATIC



The Ratfisch total hydrocarbon analyzer was adjacent to the conditioning module. The analyzer was a heated type and the sample path temperature was maintained at 160 degress C. The sample flow to the detector was about 50 ml/min. and the rest of the sample was bypassed. The monitor´s range was set at 1000 ppm and the span gas was 225 ppm methane in nitrogen. This span gas was later on replaced by 545 ppm methane in nitrogen so as to approach the more typical THC ranges observed in this study. The span gas was injected either at the analyzer or at the probe. In the first case, the gas was used to calibrate the instrument, whereas in the second case, it served to verify the operation of the sample conditioning system.

The THC measurements were registered on a potentiometric strip chart recorder with problematic Z-fold feed.

## RESULTS AND DISCUSSION

The THC system was installed on April 10, 1985 and remained on site for the next 34 days. The system was inspected daily during the first week of operation and every three to four days thereafter. The inspection consisted of the following:

a) verification of dilution system and analyzer temperatures
b) checking the zero and span values
c) checking sample back pressure
d) recalibrating the analyzer
e) checking dilution ratio by feeding span gas at the sample probe.

Maintenance was limited to changing the coarse particulate filter after 25 days of operation. However, the thermostat of the THC analyzer was repaired during the test period, resulting in 48 hrs of lost data. Another 24 hours were lost when the cylinder ran out of fuel-air. Additional data losses resulted from jammed chart paper.

In total, the THC system produced 545 hours of valid data. The operating record of the system is summarized in Table 1.

Table 1
THC SYSTEM OPERATING RECORD

| | |
|---|---|
| Valid measurement records | 546 hrs ( 67%) |
| Chart recorder malfunction | 166 hrs ( 20%) |
| Analyzer malfunction & repair | 48 hrs ( 6%) |
| Calibration | 24 hrs ( 3%) |
| Out of compressed air | 24 hrs ( 3%) |
| Maintenance | 8 hrs ( 1%) |
| TOTAL | 816 hrs (100%) |

The hydrocarbon concentrations values averaged 720 ppm and the daily averages ranged from 432 ppm to 1229 ppm. No attempt was made to relate these THC concentrations with incinerator operating conditions. The 720 ppm average is close to the 698 ppm average measured at this site in April of 1982 and significantly higher than previously recorded in May of 1979 (200-400 ppm).

The THC measurements are summarized in Table 2.

Table 2
THC MEASUREMENTS SUMMARY

| DATE | MEAS. HOURS | THC PPM AVE. | STD. DEV. |
|---|---|---|---|
| April 11 | 9 | 465 | 84 |
| April 12 | 24 | 435 | 172 |
| April 13 | 24 | 688 | 285 |
| Arpil 14 | 24 | 577 | 128 |
| April 15 | 24 | 791 | 450 |
| April 16 | 22 | 932 | 300 |
| April 17 | 22 | 785 | 329 |
| April 18 | 22 | 574 | 190 |
| April 21 | 12 | 533 | 198 |
| April 22 | 24 | 473 | 68 |
| April 23 | 22 | 619 | 126 |
| April 24 | 24 | 1035 | 142 |
| April 25 | 23 | 868 | 127 |
| April 26 | 24 | 1006 | 164 |
| April 27 | 22 | 1273 | 260 |
| April 28 | 24 | 1229 | 461 |
| April 29 | 23 | 689 | 182 |
| April 30 | 23 | 734 | 162 |
| May 1 | 7 | 432 | 110 |
| May 8 | 11 | 659 | 77 |
| May 9 | 24 | 948 | 134 |
| May 10 | 24 | 688 | 51 |
| May 11 | 24 | 465 | 113 |
| May 12 | 24 | 634 | 122 |
| May 13 | 24 | 579 | 92 |
| May 14 | 15 | 618 | 46 |
| TOTAL | 545 | - | - |
| AVERAGE | 20.9 | 720 | 176 |

The analyzer zero drift was less than 10 ppm and the span drift was negligible.

The dilution factor of the sampling conditioner was checked several times during the testing period. The dilution factor averaged 0.53 and ranged from 0.50 to 0.55, with one exception. The exception (dilution factor =0.70) was probably due to improper FID setting.

The average solution factor was the perimeter used to calculate the Table 1 concentrations. The dilution factor verifications are listed in Table 3.

Table 3
DILUTION FACTOR VERIFICATIONS

| DATE | DILUTION FACTOR |
|---|---|
| April 13, 1985 | 0.53 |
| April 14, 1985 | 0.54 |
| April 15, 1985 | 0.50 |
| April 16, 1985 | 0.51 |
| April 17, 1985 | 0.53 |
| April 18, 1985 | 0.52 |
| April 21, 1985 | 0.55 |
| April 23, 1985 | 0.50 |
| April 25, 1985 | 0.50 |
| April 26, 1985 | 0.70* |
| April 28, 1985 | 0.54 |
| April 30, 1985 | 0.54 |
| May 7, 1985 | 0.52 |
| May 8, 1985 | 0.51 |
| May 14, 1985 | 0.50 |
| AVERAGE | 0.53 |

*Recalibration made fuel-air setting too sensitive.

$$\text{Dil.factor} = \frac{\text{Meas. of span gas injected at the probe}}{\text{Meas. of span gas injected at the analyzer}}$$

## CONCLUSIONS

1. The feasability of monitoring hydrocarbon emissions at this site was demonstrated. The maintenance requirements of a THC system equipped with an adequate sample conditioner are in line with other types of continuous monitoring systems.

2. The THC monitoring system produced 546 hours of valid data (67% up-time). Unavoidable data losses, resulting from analyzer calibration, maintenance and repair, totalled 10%. Data losses caused by recorder malfunction and insufficient air supply totalled 23%, and may have been easily avoided with in-plant maintenance.

3. The hydrocarbon concentration measurements averaged 720 ppm and the daily averages ranged from 432 ppm to 1229 ppm. Some process modifications were implemented since these tests and their effect will be subject to further testing.

The co-operation between the MOE´s Source Assessment Unit and AMKO Systems Inc., has been of significant value in demonstrating the ease of obtaining valid continuous THC data from a sludge incinerator. For the first time in seven years at this site, continuous hydrocarbon emission data was realized. The success of this project was based on 1) design of an appropriate sample system and 2) an FID THC analyzer designed for this type of operation. With additional funding, daily inspection could have easily resulted in 95% up-time. It must be noted that this study has benefited AMKO by extending its application-base into this priority area while resolving a question that needed to be addressed for years.

# STUDY OF THE SPATIAL DISTRIBUTION OF THE IMPACT OF SUDBURY SMELTING EMISSIONS

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Waterloo, Ontario, Canada N2L 3G1

## ABSTRACT

The two mining and smelting operations near Sudbury, Ontario are the source of greater than half of the total $SO_2$ emissions in Ontario (Tang et al., 1984). These releases are examined to improve the understanding of the resulting areal deposition patterns. The intent of this focus is threefold:

(i) to develop contouring procedures which will allow generalization of point-specific measured information, and to indicate regions that would be impacted by various levels of remedial control technologies;

(ii) to better understand the long term deposition levels, reflecting meteorologic variability on annual and seasonal levels; and

(iii) to compare with monitoring station records to observe whether additional and/or fewer monitoring stations may be appropriate.

## 1. INTRODUCTION

This paper is a review of some of the acid rain research going on at the University of Waterloo under funding from the Ontario Ministry of the Environment and the National Scientific and Engineering Research Council. The specific focus of the Ministry funding is a two part study of the Acid Precipitation in Ontario (APIOS) Air and Precipitation chemistry network. Phase I is directed at the spatial definition of the impacts of $SO_2$ emissions from the INCO and Falconbridge smelters at Sudbury. The second phase will utilize the North American source inventory (ie. 253 point sources and 83 areal sources) in a detailed analysis of the spatial resolution provided by the APIOS network. It is felt that this work will provide useful informattion regarding the capability of the APIOS network to:

(a) accurately reflect the true spatial pattern of acid deposition over much of Ontario;

(b) identify significant changes in emissions at major sources within an established time period.

A significant portion of the work involves the development of an accurate spatial analysis technique for characterizing acid deposition patterns. Kriging, a spatial interpolation method that minimizes the interpolative error, and provides an estimate of that error, is being utilized as the tool for integrating discrete data points to a spatial field. However, the application of kriging techniques to large geographic areas is subject to error due to the distortion in projecting a spherical surface onto a square grid. Development of a modified kriging method, employing transforms of lattitude and longitude to a square grid for kriging analysis is ongoing at this time.

## 2. BACKGROUND

The two mining and smelting operations near Sudbury are the source of greater than half of the total $SO_2$ emissions in Ontario (Tang et al., 1984). Scientists had generally assumed the major environmental impact of Sudbury emissions was upon the immediate area of Northern Ontario. This assumption was accepted because deposition amounts in Sudbury were high, and it was logical to attribute the deposition to the nearby, large emission sources. However, this has been shown to not be the case. Chan et al. (1984) and Tang et al. (1984) studied deposition variability near Sudbury and throughout Ontario. Using deposition and air concentration data, and sector stratification techniques, Chan et al. concluded that very little of the sulfur compounds emitted at Sudbury are deposited close to the source (within 40 km.). Most of the deposition in this area was attributed to upwind sources. Tang et al. utilized extensive periods of smelter shutdown during 1982 and 1983 to demonstrate the upper limit of Sudbury contributions to deposition at six Ontario stations ranged from two to forty seven percent. These studies demonstrated two points:

1. most of the acid deposition around Sudbury is due to pollutants transported into the area from other sources; and
2. most of the Sudbury emissions are deposited outside of the Sudbury area.

The analysis of Sudbury emission deposition patterns to be presented below, utilize the UW-LRT Model. The model is a modified version of the Ministry LRT statistical model (Venkatram et al., 1982). Shipley (1986) modified the original model to include trajectory analysis routines in the calculation of the transfer coefficients. The dispersion function was refined further to include variable wind speed and the resultant variation in dispersion (Byrne et al., 1986). A comparison of model results to output from the more complex AES LRT model (Olson and Voldner, 1981) demonstrates the UW-LRT model is a practical, although simpler and more approximate, alternative that may be used to address certain questions.

## 3. RESEARCH PROCEDURES

In this study, the focus is on the impact of Sudbury emissions. To accomplish the objectives of this work, the following steps have been carried out:

1. Statistics on wind variability from season to season have been compiled as input for UW-LRT simulations;
2. the Sudbury impacts on eastern North America were generated;
3. Mapping of impacts - the model-defined spatial patterns will be utilized in concert with kriging techniques to define the actual area impacted by Sudbury emissions, and the variation of the impacts within that area.

The intent of this analysis is threefold:

(i) to provide generalized information regarding the improvement in environmental quality in regions impacted;

(ii) long-term impacts, reflecting meteorologic variability, will be determined. Our intent is to examine the contours arising from years for which there are recorded deposition data, the "average" year, and its associated distribution.

(iii)the deposition patterns will be compared with the monitoring stations to observe whether additional and/or fewer stations are appropriate. Several alternative tests will be utilized to determine the information returns for changes in the number of stations in the APIOS network.

It should be noted that the above pertains principally to the impacts of Sudbury. We have included in Part I only this source to assist in development of the methodology and to explore the effect of alternative approaches to contouring. In Phase II, the information will be generalized to other sources. It is only after Phase II that an assessment of the entire APIOS network will be complete.

## 4. UW-LRT MODEL

The University of Waterloo LRT Model combines a statistical trajectory routine (Shipley, 1986) with a dispersion function adapted from Ellenton et al. (1985) and a mass balance scavenging function developed by Venkatram et al. (1982). The trajectory routine utilizes wind direction and speed statistics compiled from analyzed, six hourly, upper air trajectory fields at the 850 mbar constant pressure surface. Output from the model consists of source-receptor deposition linkages (transfer coefficients). For the simulations presented here, the 1980 emission inventory for eastern North America was used for all years. The inventory contains 318 $SO_2$ sources, of which 235 are point sources and the remaining are areal sources. The emission sources were matched to the nearest of 120 wind data grid points. Multiple sources at a common grid point were summed, and treated as a single source located at an emission-weighted mean lattitude and longitude. There was no combining of point and areal sources. The end result was a pooled network of 45 point sources and 29 areal sources, for a total of 74 pollutant sources. Model simulations were run for receptor grid of 192 points over eastern North America. Modelled spatial patterns were drawn using standard contouring packages.

## 5. RECOGNITION OF EMISSION REDUCTION

A key question for managers of acid deposition monitoring networks involves the ability of the network to recognize a change in deposition levels due to discrete changes in emissions at a major source(s). Efforts to improve deposition pattern resolution involves detailed network design and station siting criteria, and well defined collection, storage and analysis techniques. However, minimization of system errors, while necessary, does not address the natural variability in acid deposition due to atmospheric processes (wind speed and direction, mixing height, precipitation, chemistry, etc.). Attempts to define the relative impact of the parameters contributing to acid deposition variability are ongoing. The opinion here is that system error is small compared to the true spatial variation in acid deposition. This view is supported in the literature (Granat, 1975). In order to recognize a change in deposition that is directly related to emission reduction, one must first be capable of manipulating the data to minimize, to a reasonable degree, the variation due to the physical processes. At that point, the principal parameter affecting deposition would be the emission variation.

The ongoing analysis of variation in wet sulfur deposition at UW theorizes that three components contribute most of the variability in acid deposition. These are: transport phenomena (ie wind trajectory and speed); deposition phenomena (principally spatial and temporal precipitation patterns); and emission variation. The UW-LRT model is felt to reflects elements of the transport phenomena and its associated variability. The effect of precipitation spatial patterns is currently being examined, for eventual comparison to analyzed spatial deposition fields. However, analysis of the rainfall and deposition fields will not be completed until the distance transform routines have been verified.

Several studies (Chan et al., 1982; Tang and Chan, 1985) have attempted to define the regional impact of Sudbury emissions by comparing deposition data collected during extensive smelter shutdowns to data collected during normal operations. These studies found a high degree of deposition variability, but with careful analyses, they were able to establish that most of the deposition of wet sulfur in the Sudbury area is due to emission from other sources. Of interest in this study is to determine the extent to which adjustments of the two data sets (operational and non-operational) to minimize other forms of variability would improve the understanding of the deposition patterns. The following discussion demonstrates how LRT variability effected the Sudbury area deposition during the shutdown period.

The INCO and Falconbridge smelters contribute 812 and 123 k tonnes of $SO_2$ , respectively, to the emission levels at Sudbury. Both plants were shut down (with minor exceptions) from July, 1982 to January, 1983, and INCO remained shutdown for the first quarter of 1983. Figures 1, 2 and 3 are UW-LRT simulations of wet $SO_4$ deposition for the summer (July - September), and fall (October - December), 1982, and the winter (January - March), 1983. A background level of 1.5 kg. $SO_4$ / ha is included. The simulated patterns assume all sources emitted at 1980 levels, therefore, emission variability is nil. This assumption is the only possible approach since emission inventories do not exist for other years. The contours reflect the deposition that would have occurred, given the variability in trajectory statistics for each quarter. Examination of the figures indicates the deposition rate is greatest in summer and smallest in winter. This is not unexpected, given the model incorporates the effects of transport winds. Typical summer conditions in eastern North America involve slow air movements associated with stagnant high pressure cells of a sub-continental scale. Dispersion is less effective with low wind velocities, and pollutant concentrations would tend to increase to higher levels. Winter conditions involve frequent storms associated with the intensified westerly flow that dominates weather conditions. Wind levels are generally higher; consequently, pollutants are dispersed quickly, and transported out of the deposition area in less time. The Fall of 1982 (Figure 13) represents a transition phase.

Figures 4, 5 and 6 provide estimates of the magnitude of deposition change that may be expected due to the Sudbury shutdown. For example, for Summer 1982, the net effect on the APIOS network would have been in the range of 0.6 to 1.4 kg. $SO_4$ / ha. These small, net values would be very difficult to recognize in a deposition field with a high variation, which is in fact, the case.

Simulations of the eastern North American deposition for the shutdown seasons are provided in Figures 7, 8 and 9. These figures are identical to Figures 1, 2 and

3 except that the Sudbury emissions were set to 0. Preliminary comparisons of the figures reveals little. However, overlay comparison demonstrates a definite and significant shift in the isopleths as a direct response to Sudbury emissions. Figure 10 demonstrates this shift for three prominent contours. The change, though undeniable, is likely not statistically significant. Current efforts are being directed at defining statistical measures that will detect this type of variation. However, development of a suitable test for recorded data is dependent on the development of an accurate kriging routine, together with the associated projection transforms.

## 6. MULTI-YEAR WIND VARIABILITIES AND DETECTION OF SUDBURY SHUTDOWNS

Attempts to compare the deposition recorded during smelter shutdown periods with periods of operation are based on the assumption that the deposition is strongly related to emission levels. This is true for the macro scale only. The impact of individual sources on receptors within a reasonable proximity may not be sufficient to outweigh the variation due to other factors. The following example illustrates this phenomena. The UW-LRT model was used to simulate the quarterly depositions for the years 1980 through 1984. The deposition patterns reflect the wind speed and direction variability for each season. Therefore, the variation in location of any isopleth over the total period (for any season) reflects the deposition variability due to wind variation. Figures 11, 12 and 13 present this variation for an equivalent contour for the summer, winter and fall seasons, respectively. Consider Figure 11; in Summer 1982, one expects the deposition in the Sudbury area to be lower due to the shutdown. However, the location of the 6 kg isopleth indicates that deposition would have increased in lattitude from "average" conditions because the wind conditions have effectively shifted the sub-continental deposition pattern northward. This change should be accounted for in attempts to identify deposition reductions due to the shutdown. The shutdown and deposition shift effectively 'cancelled' one another. This effect is even more pronounced for winter, 1983 (Figure 12), where the 4.5 kg contour is much further north than those of other years.

## 7. CONCLUSIONS AND FUTURE WORK

Analyses of the principal factors contributing to variability in acid deposition are ongoing. A LRT Model that reflects transport variability has been developed. Spatial analysis techniques to analyse rainfall and deposition patterns are currently under development. The rainfall spatial patterns will be utilized to determine the net effect of rainfall variation on acid deposition. Refining of the analysis will be carried out while defining the spatial patterns attributable to Sudbury emissions. Phase II will involve applying the analysis to the North American situation, with a specific intent of determining the current and potential sensitivity of the APIOS network to changes in acidic emissions.

## 8. ACKNOWLEDGEMENTS

The authors wish to thank Maris Lusis, Walter Chan and Al Tang of the Air Resources Branch for valued input. Marvin Olson, AES Canada, kindly provided the wind field data used in this study.

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**Figure 1** SPATIAL DEPOSITION - SUMMER 1982. 74 SOURCES
UW - LRT SIMULATION

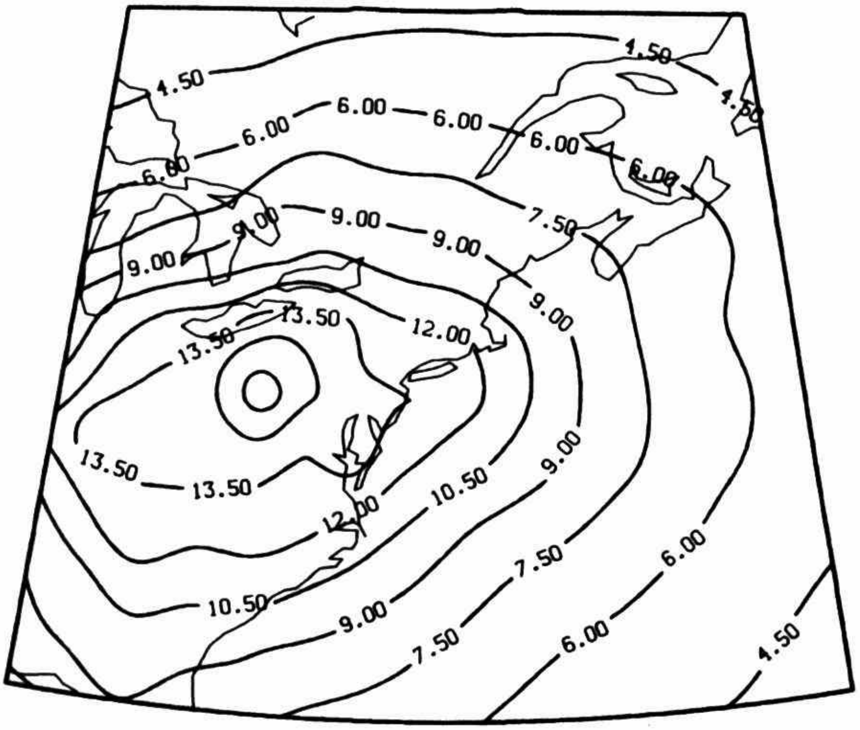


**Figure 2** SPATIAL DEPOSITION - FALL 1982. 74 SOURCES
UW - LRT SIMULATION

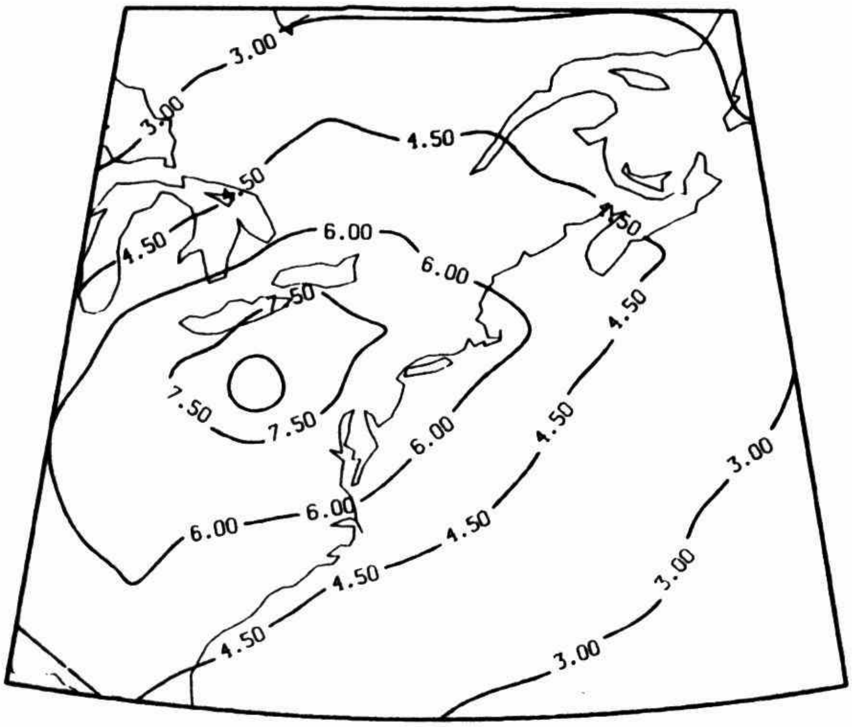
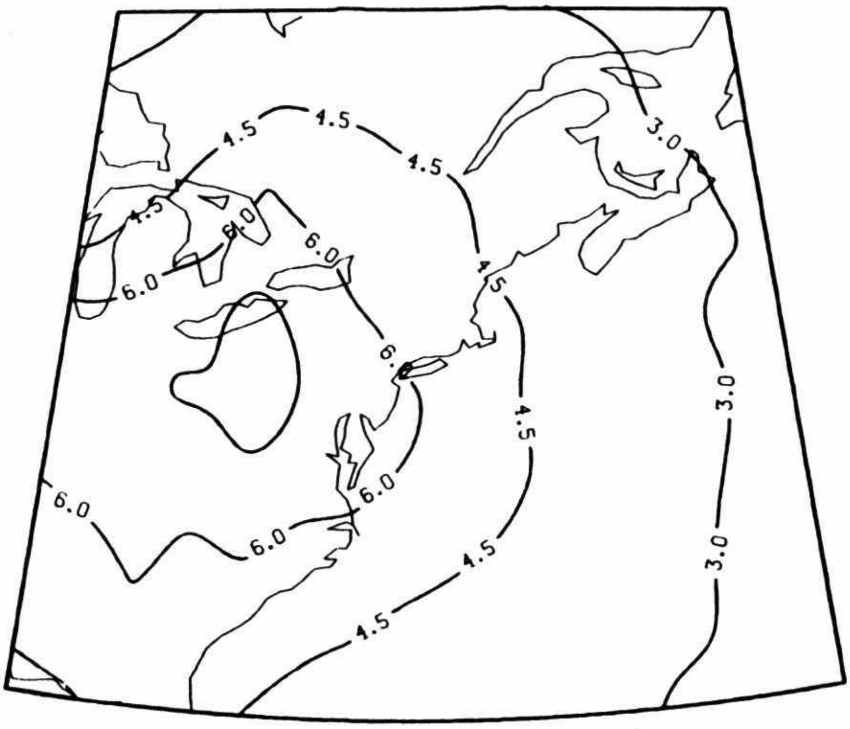


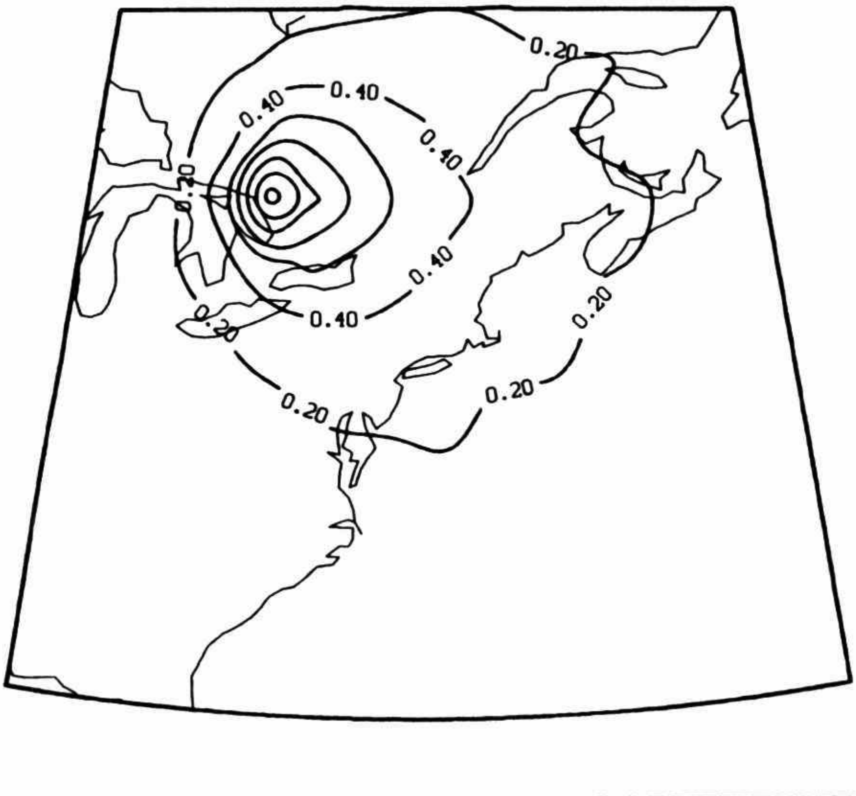
**Figure 3** SPATIAL DEPOSITION - WINTER 1983. 74 SOURCES

UW - LRT SIMULATION



SPATIAL DEPOSITION - SUMMER 1982. SUDBURY SOURCE

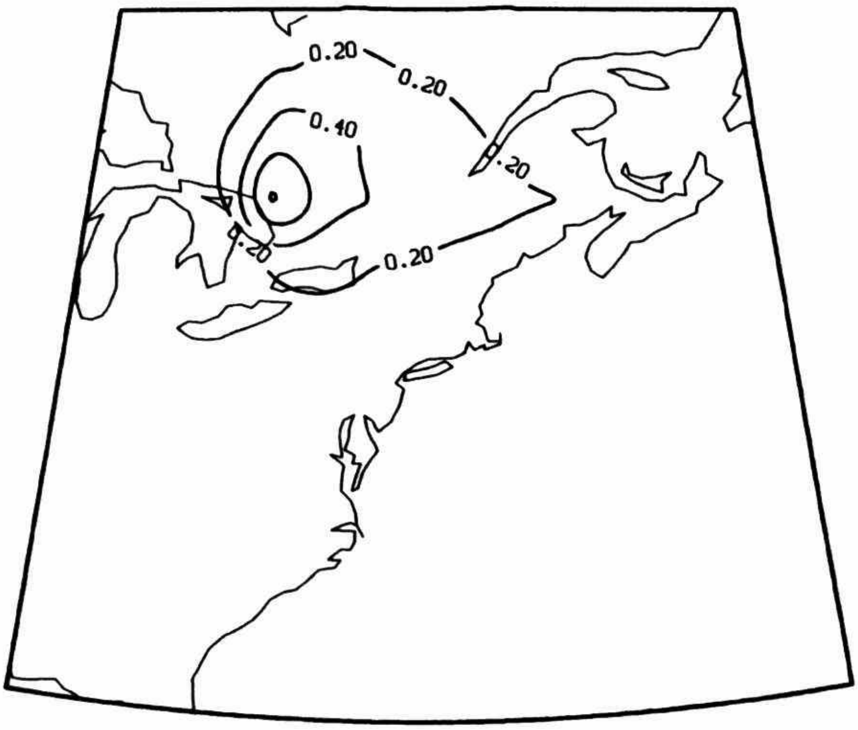
**Figure 4** UW - LRT SIMULATION



SPATIAL DEPOSITION - FALL 1982. SUDBURY SOURCE

UW - LRT SIMULATION

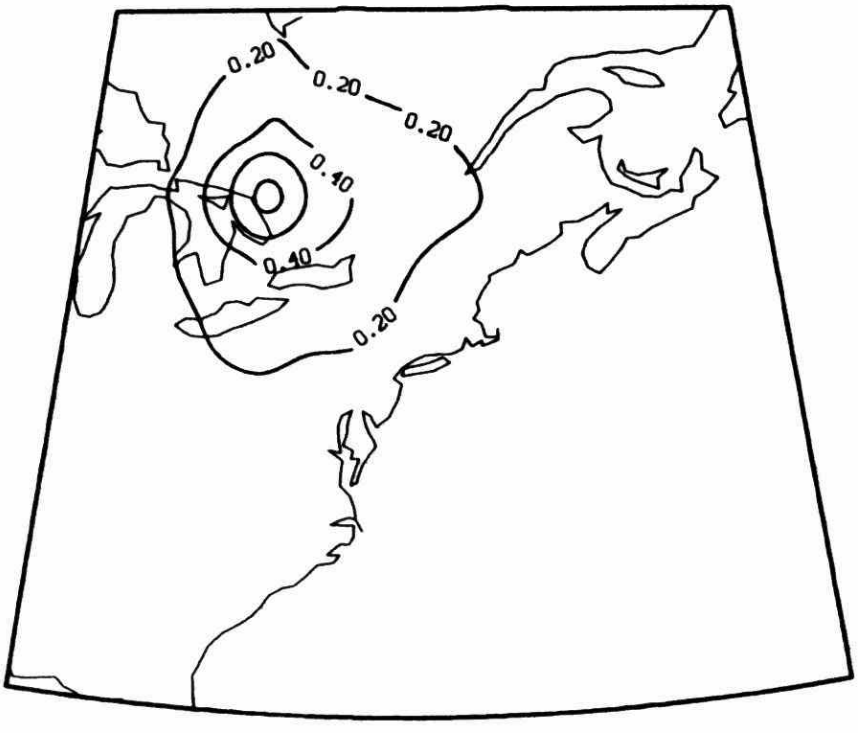
**Figure 6**



SPATIAL DEPOSITION - WINTER 1983. SUDBURY SOURCE

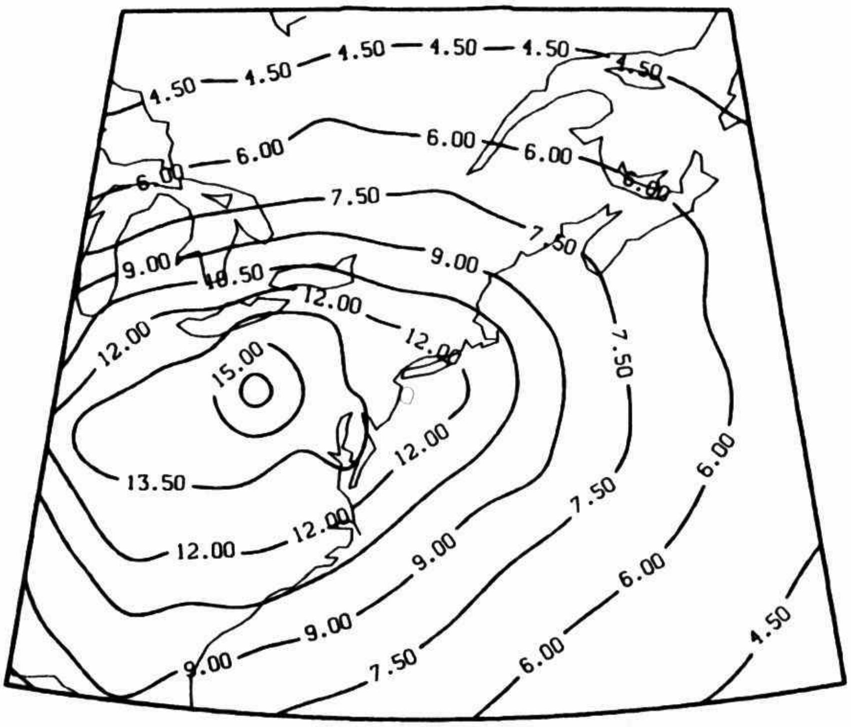
UW - LRT SIMULATION

**Figure 5**



SPATIAL DEPOSITION - SUMMER 1982. 73 SOURCES
(SUDBURY SOURCE OMITTED -- SMELTER SHUTDOWN)

**Figure 7**



SPATIAL DEPOSITION - FALL 1982. 73 SOURCES
(SUDBURY SOURCE OMITTED -- SMELTER SHUTDOWN)

**Figure 8**

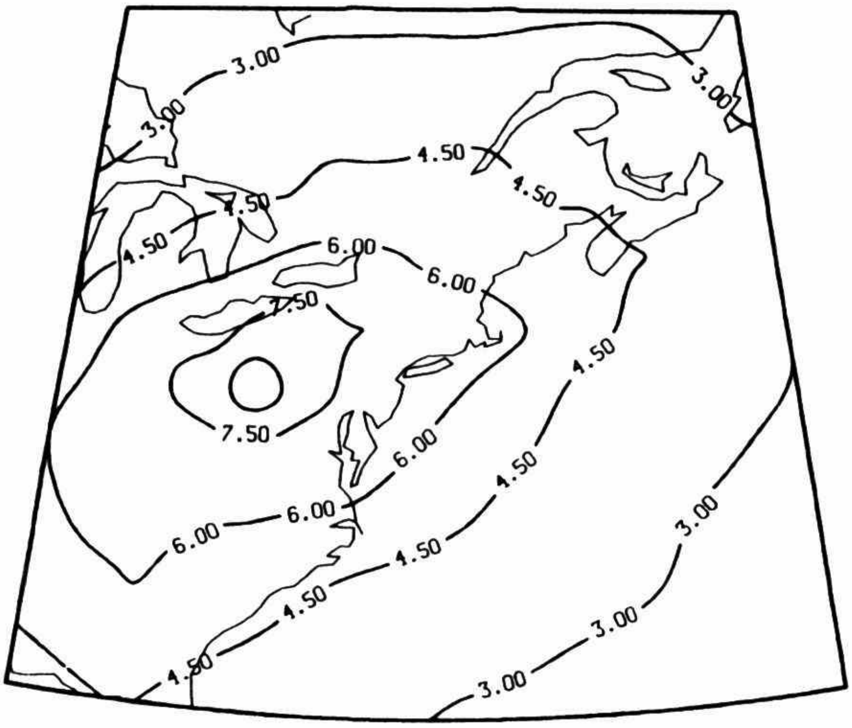


**Figure 9** SPATIAL DEPOSITION - WINTER 1983. 73 SOURCES

(SUDBURY SOURCE OMITTED -- SMELTER SHUTDOWN)

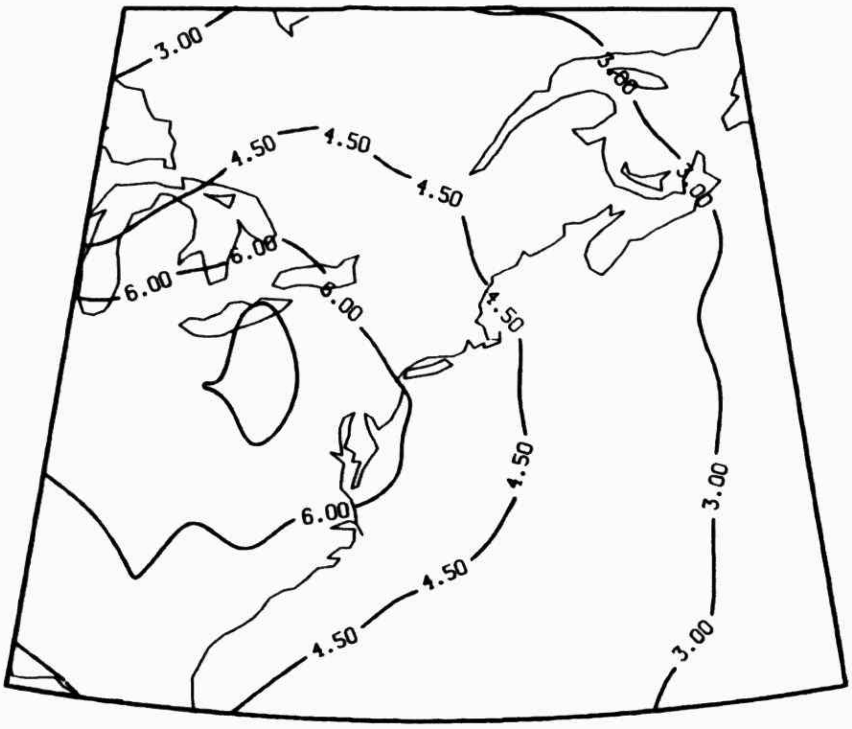
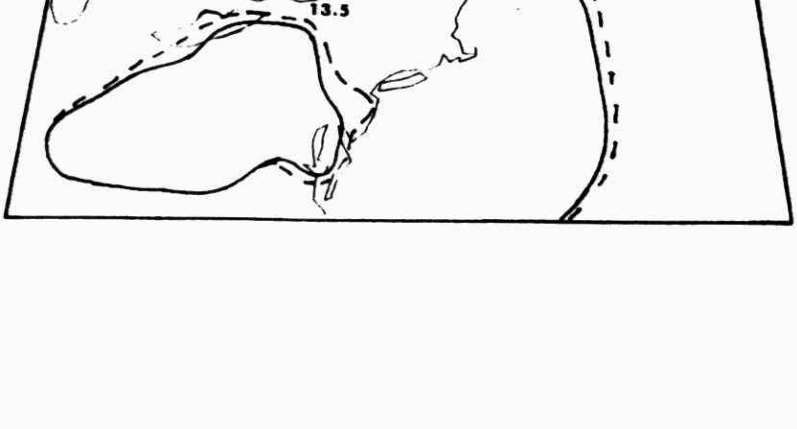


Figure 10
Composition of equivalent contours from figures 1 and 7.



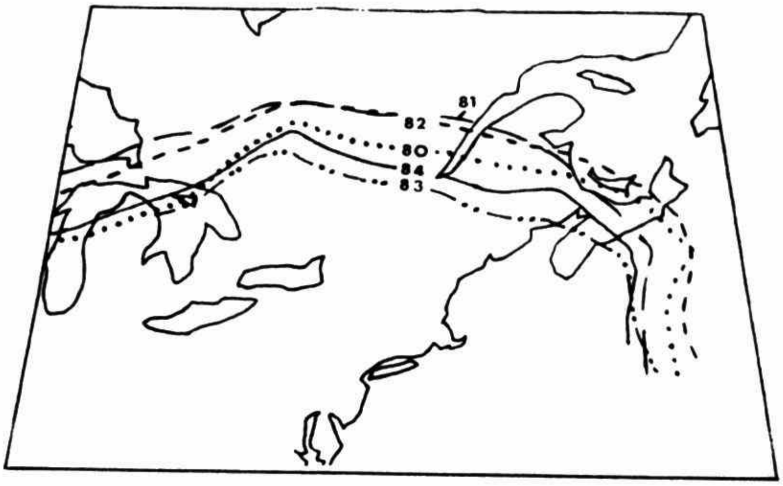


Figure 11
Locations of the 6.0 kg deposition contour for the summer season, 1980-84; UW-LRT Simulation.
(Units: kg $SO_4$/ha)

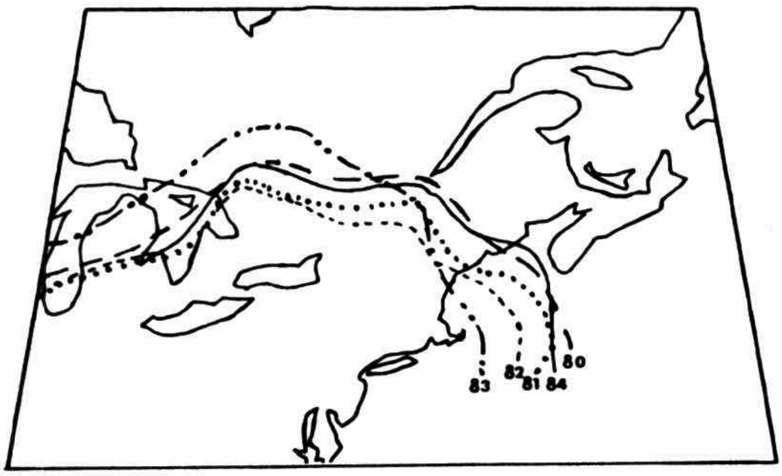


Figure 12
Locations of the 4.5 kg deposition contour for 1980-84, winter season; UW-LRT simulation.
(Units: kg $SO_4$/ha)

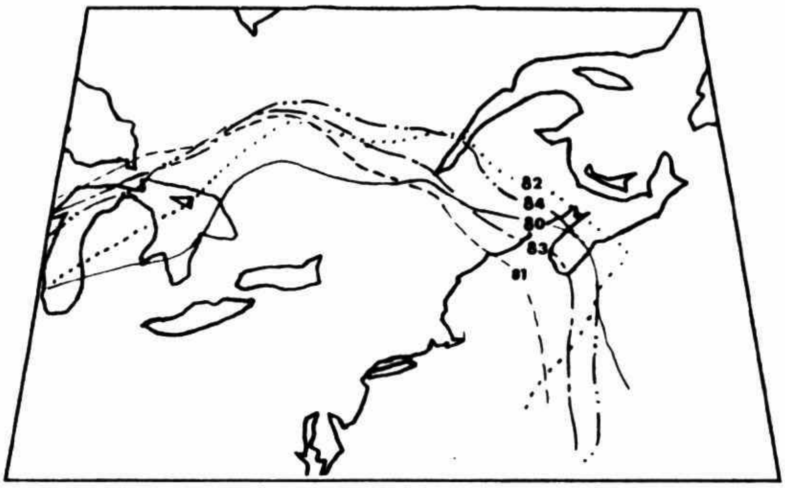


Figure 13
4.5 kg deposition contours for 1980-84, fall season; UW-LRT simulation
(Units: kg $SO_4$/ha)

# Characterization of Building Wake Effects at Darlington Nuclear Generating Station using Sulfur Hexafluoxide (SF6) as a Tracer

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Etobicoke

## ABSTRACT :

The dispersion of materials released into the atmosphere from Darlington Nuclear Generating Station was studied using tracer gas techniques. Sulfur Hexafluoride was released from the vaccum building and the reactor building stack No.2. Tracer concentarions were measured dowinwind on an array of fixed samplers at the station boundary and by mobile monitor at various points between the release point and the site boundary. Simaltenoeus meteorological measurements were carried using acoustic sounders, anemometers and pibal-minisonde flights. Approximately forty hours of data were collected under different meterological conditions. Figure 1 shows the genral layout of the area and the sampling arcs.

Prliminary analysis of the data indicates that the plume centreline dilution factors at the site boundry are not strongly dependent on the atmospheric conditions, probably because the effects of building wake and complex terrain dominate. It was also observed that the tracer was entarined into the building wake on almost all occasions, leading to high ground level concentrations immediately downwind of the release. Site boundary dilution factors ranged from $8.2 \times 10^{-7}$ s/m$^3$ to $1.1 \times 10^{-5}$, a variation of only an order of magnitude.

## INTRODUCTION :

Air flow aound buildings and other obstructions is quite complex and is chracterised by the presence of a wake region and one or more zones of recirculating secondary flows. Substantial literature exist on the theoratical and wind tunnel investigation of such flows. However there have been very few full scale experiments, primarily due to limitations in the istrumentations for characterizing the flow field and the logistics involved.

Dispersion of material emitted from the top a large structure is greatly influenced by the wake generated by the structure. Ontario Hydro operates two nuclear power generating stations located at Pickering Bruce and the third one at Darlington is under construction. Most of the emissions at these stations emanate from rooftop stacks and thus are affected by the building wake. In addition to the building wake effects all sites have reasonably complex topography. This paper describes a filed study which was carried out at Darlington to delineate the effects of building wake and topgraphy on dispersion of materials released from vaccum building and the reactor building.

Darlington Nuclear Generating Station is located on the northern shore of lake Ontario and is about 60 km east of Toronto. The main structure is about 600m (L) x 150m (W) x 50M (H). There is separate vaccum building which is circular and is also about 50m high. Topography around the buildings is quite complex. It rises sharply about 500 m from the building and goes up to about 60 to 70m height. On the west side there exists a large mound of excavated soil to the east there is undulating ground. The site boundary ranges from about 1 km to 2 km from the station depending on the direction.

## EXPERIMENTAL METHODS :

Sulfur Hexafluoride (SF6), non toxic, colourles odourless gas was used as a tracer. The gas was released at a constant rate from a pressurized tank, using a regulator and a flow controller, through a 1/4 in. copper tube. Flow rate was typically 10 litres/min and was accurate to within one percent.

Concentraions of SF6 were measured using electron capture gas chromatography. The minimum detectable concentration was better than 5 pptv (parts per trillion by volume). At a concentration of 100 pptv, measurements were reproducible to within ten percent. Backflush arrangement was used to prevent oxygen from reaching the detector. This helped in keeping tha sample turnaround time to around two minutes.

Samplers were deployed along a fixed sampling arc, depending on the wind direction. The sampling arcs were matked at 100 m interval to facilitate in the deployment. Selected onsite roads were also marked at 50 or 100 m intervals to allow sampling of plume at shorter travel distances. Thirty sequential samplers constructed by the Research Division of Ontario Hydro were deployed along the site boundary so as to intercept the expected plume postion given the prevailing wind direction. Four sequential samples were taken with these samplers and one hour averaging time was always used.

In addition to the fixed samplers, a mobile SF6 sampleing system was setup by installing a GC in a avn. This enabled us to obtain instanteneous values of plume concentration. The mobile sampler was primarily used to define the plume profile near the release point to obtain information on the degree of plume entrainment and mixing due to the building wake. Some profiles were also taken along the site boundary for comaprison purposes.

A network of meteorological instruments was deployed to chracterise local atmospheric conditions during the experiments. The network consisted of two Bendix Frieze anemometers mounted at about 8.5 m, two monostatic acoustic sounders and a doppler acoustic sounder. In addition to onstie meteorological measuremnets, one acoustic sounder and a anemometer were installed about 6 km inland. Pilot balloon minisonde flights were also flown during most of the experiments. Data were recorded two additional on site tower whenever available. During the first phase of the experiment uvw anemometers were installed but dur to a storm they were damaged and only a limited data set were collected.

## DISCUSSION OF RESULTS :

From Nov. 13, 1985 to June 16, 1986 eleven days were selected for the tracer release. A complete analysis of the data is underway and here we are presenting prelimanry results for Nov. 13, 18, and Dec. 2 1985.

### Nov. 13, 1985:

On this day the winds did not turn onshore as anticipated so no data were collected on the asmpling arcs. Mobile sampling was carried out along the south access road that runs between the vaccum building and the lake. The plume was observed to be well mixed (Fig. 2). A maximum concentartion of 28.9 ppbv at ground level was seen at about 230 m from the release point. This coreesponds to a dilution factor of 3.5 x $10^{-4}$. The plume also appears to consist of two components; a sharply peaked component of about 200 m measured width which corresponds to about 100 m plume width when corrected for of centre projection, and another much broader component of 2 to 3 times greater lateral dimension and of 10 to 100 times lower concentration. The fact that the two subsequent traverses gave closely matching profiles indicates that this dual mode structure is not an artifact of slowly changing wind direction , but may be due to the different effects of the vaccum and powerhouse building wakes.

### Nov. 18, 1985 :

Mobile monitoring indicated a sharply defined plume within a few hundred meters of the powerhouse, clearly shown in the near lateral profile shown in Figs. 3 to 5. The latter profile was taken at about 200 m from the edge of the building and about 330 m from the release point. The winds were blowing almost parallel to the lenght of the building.

The apparent width of the plume (approximately 100 m) is close to the width of the building (about 140 m).

### Dec. 2, 1985 :

Very high winds prevalied durind the entire sampling period. obile monitor data showed large fluctuations in concentartion with time at a fixed point. Fixed samplers on the EE arc showed a plume of about 200 to 250 m width and a centreline dilution factor between $8.9 \times 10^{-7}$ and $1.7 \times 10^{-6}$. After release shutdown the concentration near the plume centreline on arc `c` appeared to decrease with a time constant of about 3 mins (Fig. 9).

## DISCUSSION :

It was observed that the site boundary dilution factors (ratio of air concentration to the release rate) are not very senstive to the atmospheric conditions. In fact the dilution factors observed during the stable conditions are less than those observed under neutral or near neutral condtions. As expected, the tracer was inavariablly entrained in the building wake.The plume width always corresponded closely to the building dimensions. These are only preliminary findings. Further data analysis is underway to quantatively delineate the effects of building wake on the dispersion chracteristics of the Darlington Nuclear Generating complex.

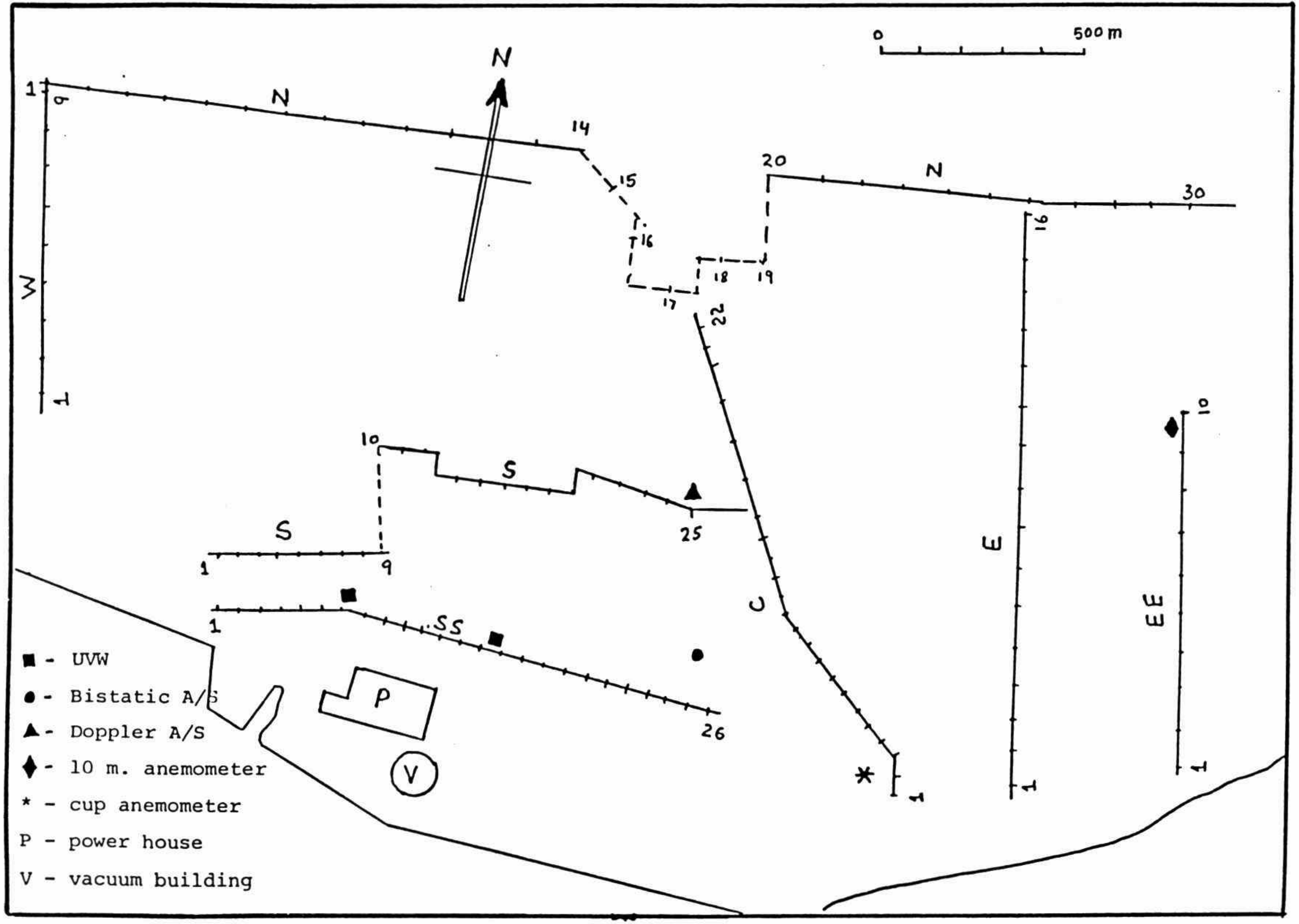

Fig.1 : Layout of Dailington Building Wake Experiment

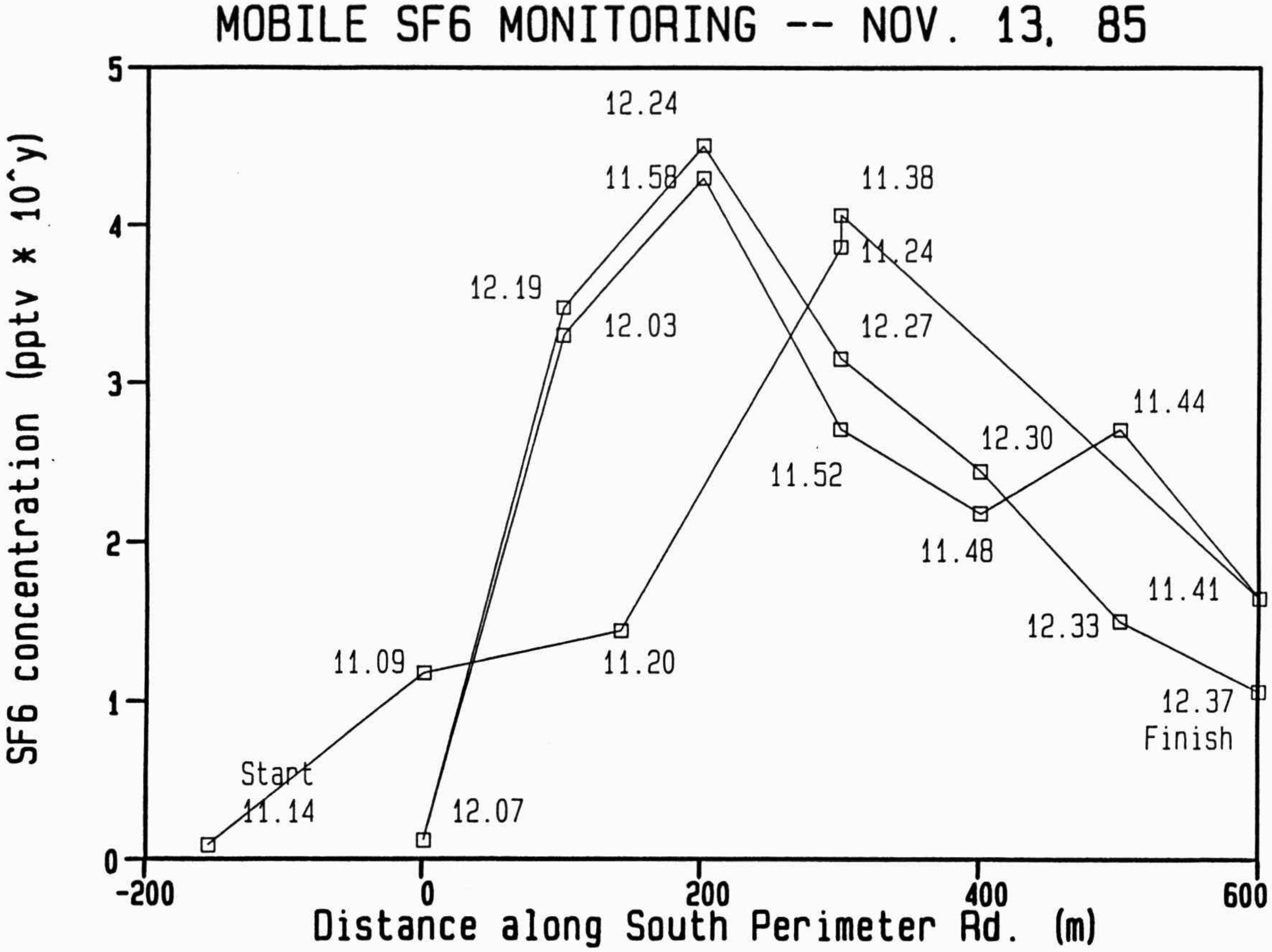
MOBILE SF6 MONITORING -- NOV. 13, 85
SF6 concentration (pptv * 10^y)
Distance along South Perimeter Rd. (m)
0
1
2
3
4
5
-200
0
200
400
600
Start
11.14
11.09
11.20
11.24
11.38
11.44
11.41
11.52
11.48
11.58
12.03
12.07
12.19
12.24
12.27
12.30
12.33
12.37
Finish

Fig. 2

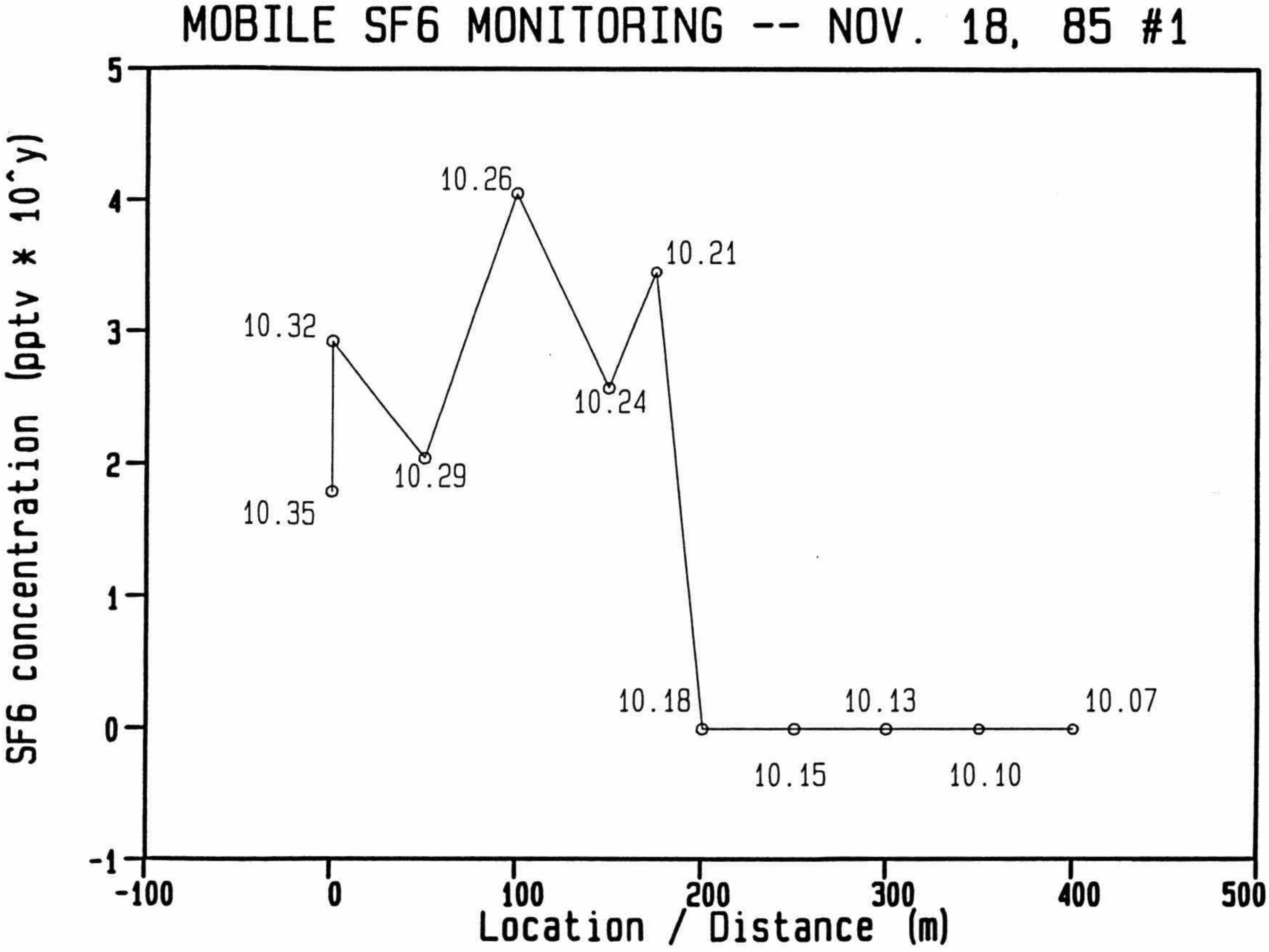

Fig. 3 : Sampling arc SS

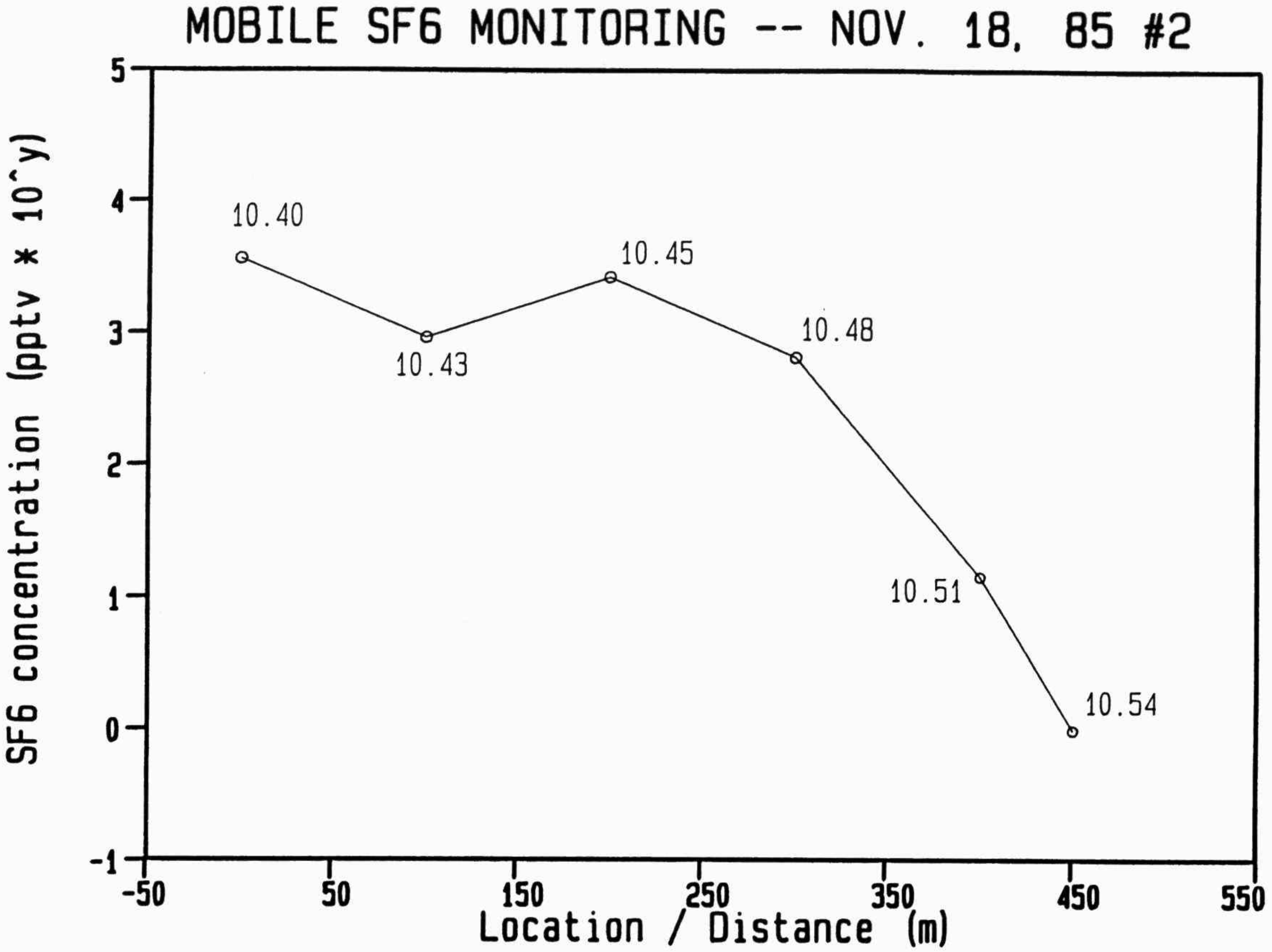

Fig. 4 : Sampling arc S

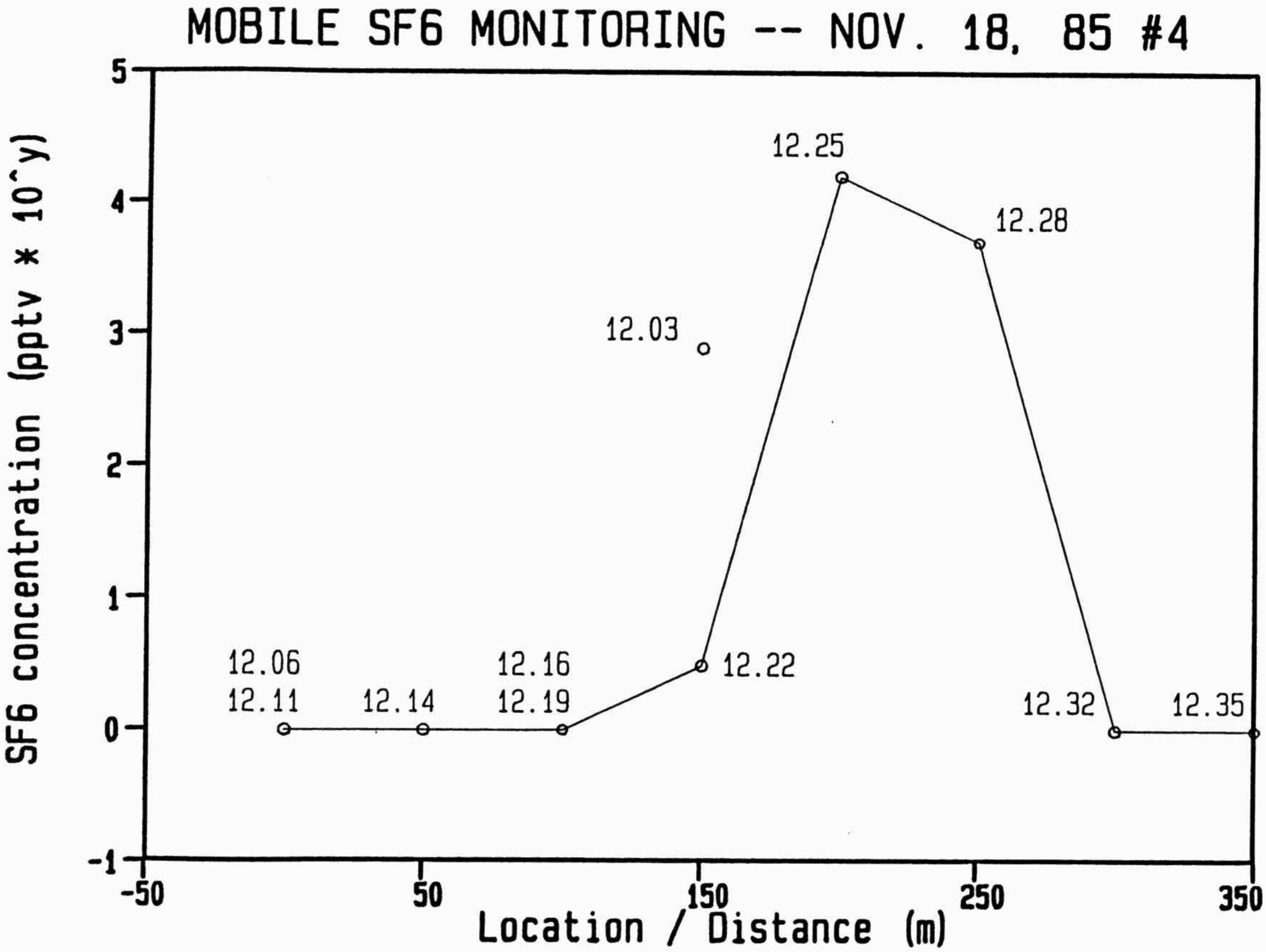

Fig. 5 : Sampling arc S. Fence

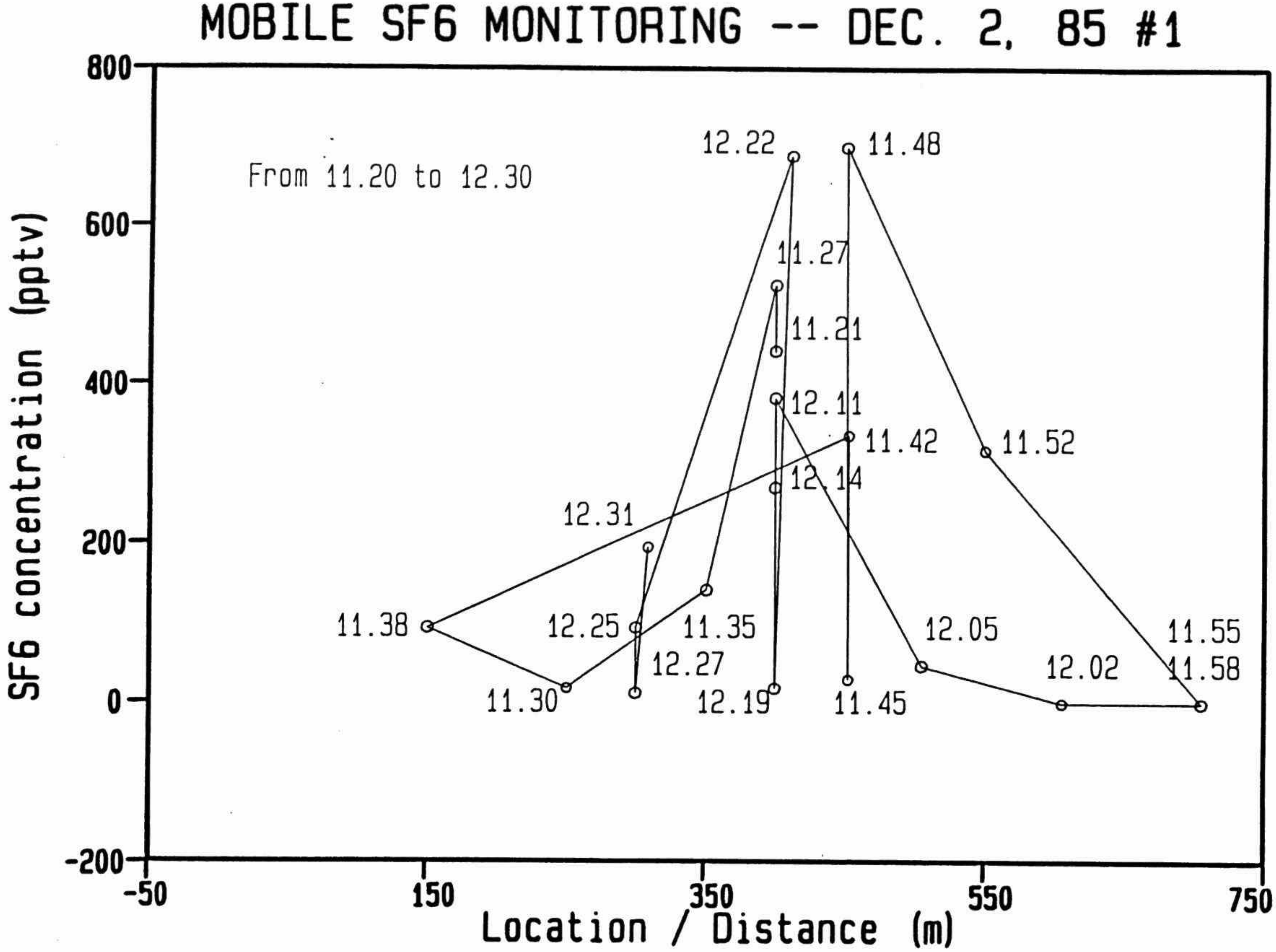

Fig. 6 : Sampling arc C

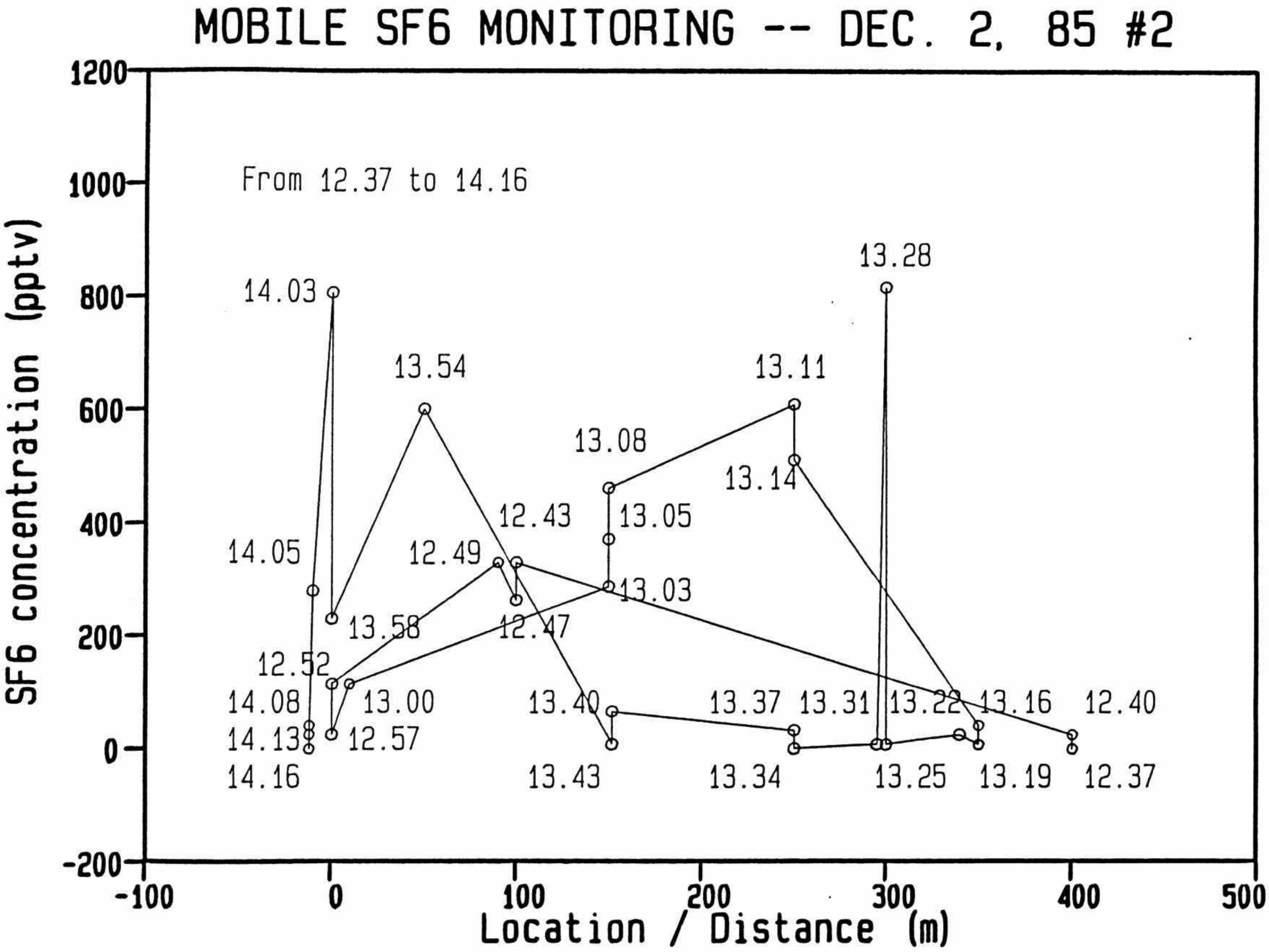

Fig. 7 : Sampling arc C

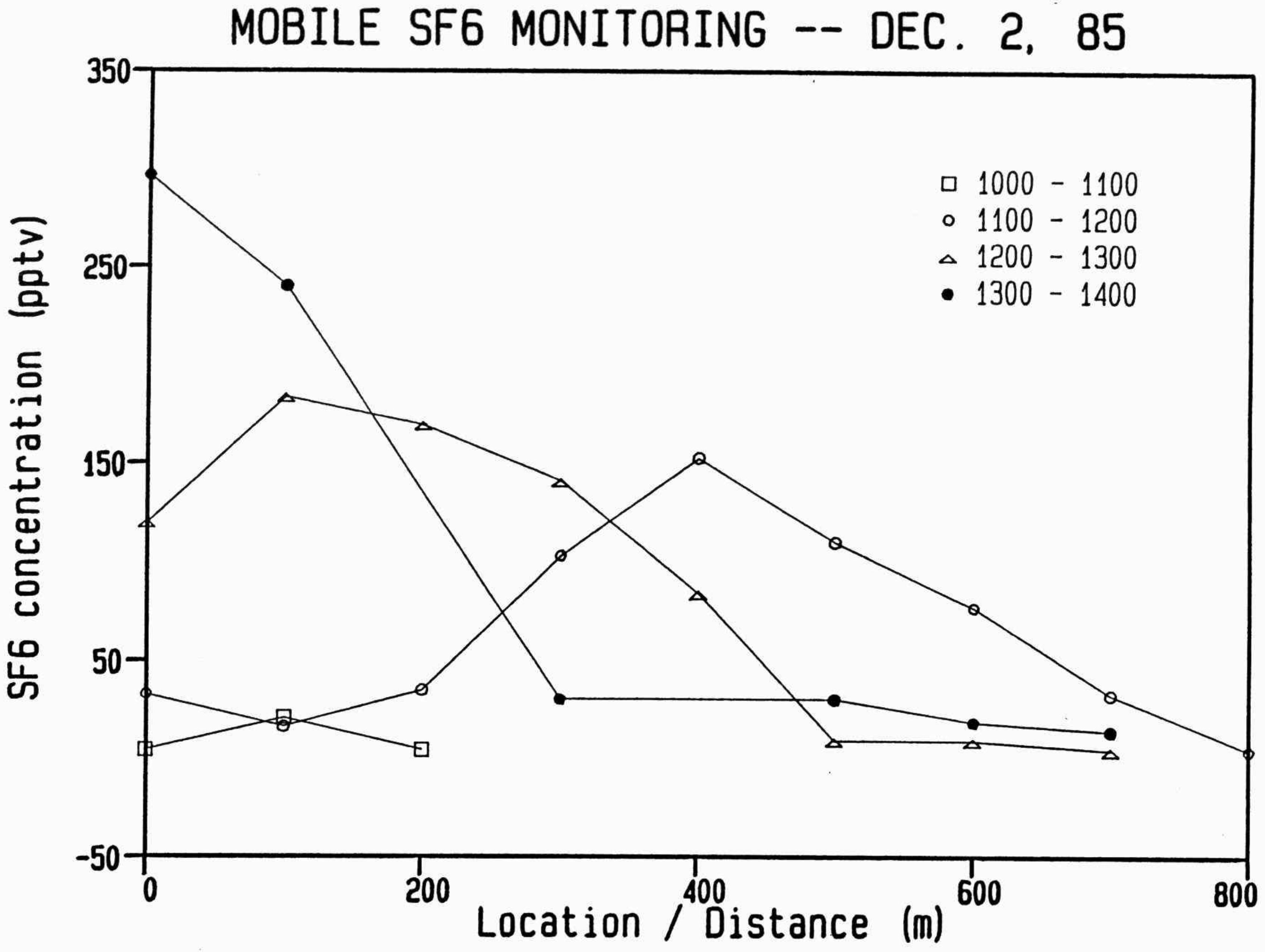

Fig. 8 : Sampling arc EE

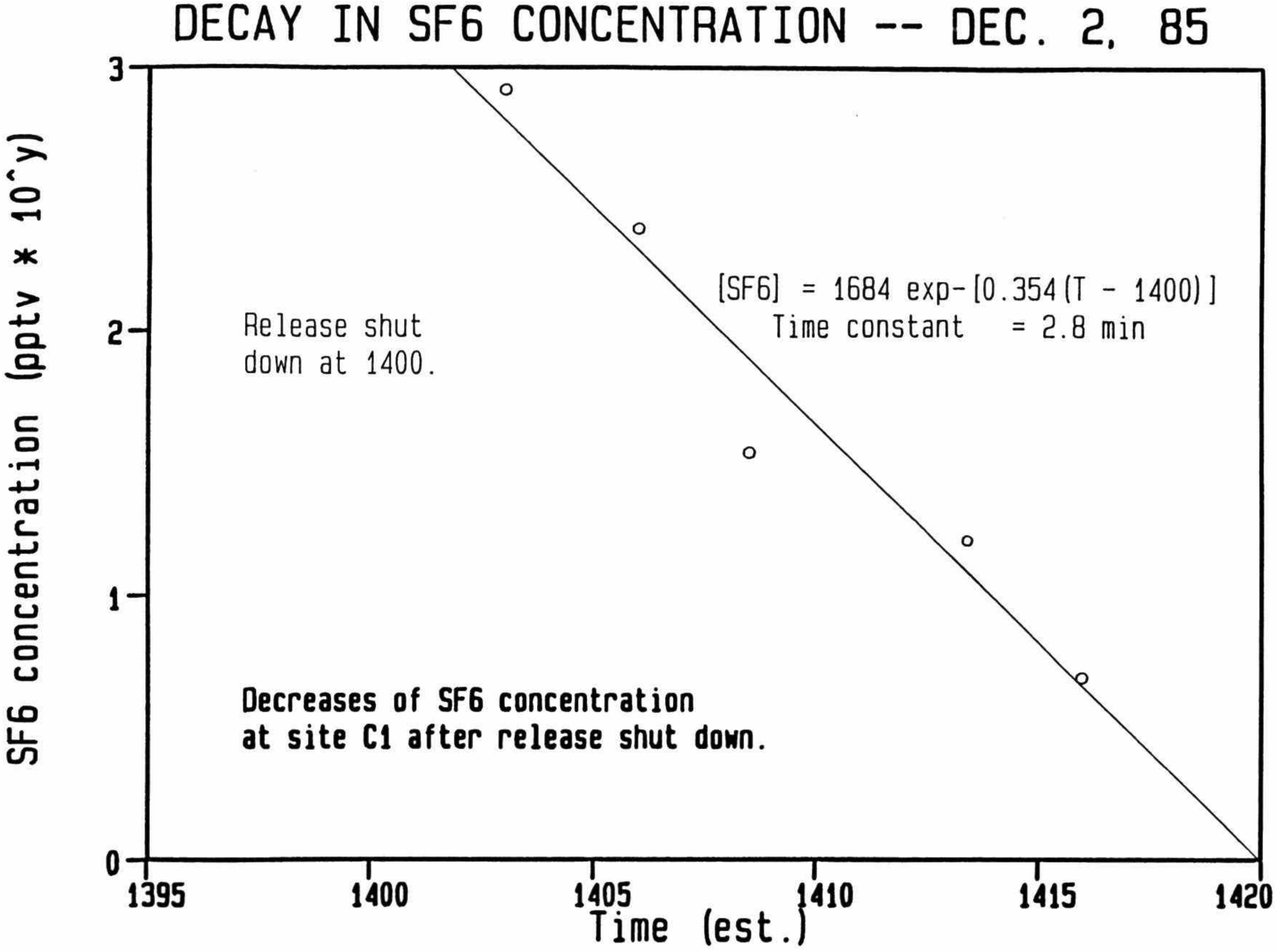
DECAY IN SF6 CONCENTRATION -- DEC. 2, 85
SF6 concentration (pptv * 10^y)
3
2
1
0
1395
1400
1405
1410
1415
1420
Time (est.)
Release shut down at 1400.
[SF6] = 1684 exp-[0.354(T - 1400)]
Time constant = 2.8 min
Decreases of SF6 concentration at site C1 after release shut down.

Fig. 9

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